

## TITLE OF THE INVENTION

IMMUNOMODULATING COMPOSITIONS, USES THEREFOR AND PROCESSES FOR THEIR PRODUCTION

## FIELD OF THE INVENTION

5 [0001] THIS INVENTION relates generally to modulation of immune responses. More particularly, the present invention relates to the use of at least one set of peptides in compositions and methods for modulating an immune response to one or more polypeptide antigens. In certain  
10 embodiments, the sequences of a respective set of peptides are derived in whole, or in part, from a single polypeptide antigen. Individual peptides of a respective peptide set comprise different portions of an amino acid sequence corresponding to a single polypeptide antigen and display partial sequence  
15 identity or similarity to at least one other peptide of the same set of peptides. The invention also extends to methods of using such peptides in a range of preventive, diagnostic and therapeutic applications. Additionally, the invention relates to the use of uncultured antigen-presenting cells or their precursors, which have not been subjected to activating conditions, and which have been  
15 contacted with an antigen, in methods and compositions for modulating an immune response in a recipient of those cells.

[0002] Bibliographic details of various publications numerically referred to in this specification are collected at the end of the description.

## BACKGROUND OF THE INVENTION

20 [0003] Since its discovery almost 20 years ago, the human immunodeficiency virus type-1 (HIV-1) has claimed more than 22 million lives and is continuing to devastate communities worldwide (1). Forty-two million people are currently living with HIV-1 and, despite efforts to modify high-risk behaviour, an estimated 5 million new infections occur yearly (2). Similarly, Hepatitis C virus (HCV) and Hepatitis B virus infections result in chronic liver damage and hepatocellular damage in millions  
25 of people worldwide. Safe and effective preventative or therapeutic vaccines for these viruses are desperately needed. Additionally, it is now believed that immune protection from, or clearance of, many cancers requires specific T cell responses.

[0004] The elimination of persistent intracellular pathogens such as replicating viruses generally requires the mobilisation of cell-mediated immunity (CMI). CD8+ cytotoxic T lymphocytes (CTL) are  
30 the primary effector cells of CMI; they kill viral-infected cells by recognising viral peptides presented on the cell surface in the context of MHC class I molecules. Prior to the appearance of virus-specific antibodies, a robust HIV-1-specific CTL response temporally correlates with reduced viremia during the acute stage of HIV-1 infection (3, 4). Furthermore, strong CTL responses are associated with reduced HIV-1 viremia during chronic infection (5, 6), whereas a decline in HIV-1-specific CTL is

linked to rapid progression to AIDS (4, 7-9). Similarly, clearance of HCV infections is generally thought to be assisted by virus-specific T cell responses.

5 [0005] There are no effective vaccines against HIV-1, HCV or cancers. Early HIV-1 vaccine strategies were based on whole-inactivated virus and recombinant structural proteins such as the envelope (*env*) glycoprotein. Non-human primate models revealed only limited strain-specific protection by these vaccines against pathogenic simian immunodeficiency virus (SIV) and highly pathogenic SHIV (SIV-HIV-1 chimeric) challenges (10-13). The first human phase III trials also failed to show efficacy (14).

10 [0006] Particle- and recombinant whole protein-based vaccines, although safe, favour the generation of antibodies that are insufficient for protection against many chronic viral pathogens. Alternatively, intracellularly expressed antigens are subsequently more likely to induce CTL responses. Live-attenuated viruses generate potent cell-mediated immunity (CMI) responses, however their clinical safety is of concern (15). Consequently, much focus has shifted toward genetically engineered vectors (such as DNA plasmids and poxviruses) expressing HIV-1/SIV genes (such as *env*,  
15 *gag* and *pol*) or HCV genes (16).

[0007] It is not known which immune-target antigens are protective, but a large breadth of T cell responses has been shown to reduce the opportunity for viral escape mutations to arise (17). It is this large breadth of potential epitopes, however, which renders the construct of large vectors frequently difficult and as well as being complicated by potential safety issues. Concerns have been raised about  
20 the potential ability of DNA vaccines to integrate with host DNA, as well as the safety of viral vector vaccines in immunocompromised hosts. These represent the significant regulatory hurdles for these recombinant vaccines.

[0008] Also, despite significant advances towards understanding how T and linear B cell epitopes are processed and presented to the immune system, the full potential of epitope-based  
25 vaccines has not been fully exploited. The main reason for this is the large number of different T cell epitopes, which must be identified for inclusion into such vaccines to cover the extreme human leucocyte antigen (HLA) polymorphism in the human population.

[0009] Infusion of whole antigen-pulsed or single epitope-pulsed cultured antigen presenting cells (APC) has previously been reported to be immunogenic in mouse models (22-27). However,  
30 other reports in inbred mouse models suggest the infusion of cells pulsed with single peptides may even be tolerogenic (induces a state of tolerance to the antigen which would be counterproductive for a vaccine) (28-31).

## SUMMARY OF THE INVENTION

[0010] The present invention discloses the discovery that autologous cells, which have been contacted with overlapping peptides of a viral polypeptide antigen of interest produce a strong immunogenic response in an outbred population that protects against subsequent viral challenge. The present inventors propose that similar protective responses would be achieved using systemic administration of the overlapping peptides *per se*. The use of multiple overlapping peptides provides several advantages, including reducing the emergence of escape mutants and the facile production of peptide-based immunogenic compositions without prior knowledge of any epitopes. In this regard, the sequence overlap between peptides reduces or prevents loss of potential epitopes, which broadens the immunological coverage of the composition to cover potentially the diversity in the major histocompatibility complex (MHC) across an outbred population.

[0011] Accordingly, in one aspect of the present invention, there is provided at least one set of peptides for modulating an immune response to one or more polypeptides of interest. Individual peptides of a respective set comprise different portions of an amino acid sequence corresponding to a single polypeptide of interest (e.g., particular pathogenic regions of a polypeptide), and display partial sequence identity or similarity to at least one other peptide of the same set of peptides. In certain embodiments, at least 2, 3, 4, 5, 6 or 7 sets of peptides are employed, wherein peptide sequences in each set are derived from a distinct polypeptide of interest.

[0012] The partial sequence identity or similarity is typically contained at one or both ends of an individual peptide. Suitably, at one or both of these ends there are at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 contiguous amino acid residues whose sequence is identical or similar to an amino acid sequence contained within at least one other of the peptides.

[0013] In certain embodiments, the peptide is at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30 amino acid residues in length and suitably no more than about 500, 200, 100, 80, 60, 50, 40 amino acid residues in length. Suitably, the length of the peptides is selected to enhance the production of a cytolytic T lymphocyte response (e.g., peptides of about 8 to about 10 amino acids in length), or a T helper lymphocyte response (e.g., peptides of about 12 to about 20 amino acids in length).

[0014] In certain embodiments, the peptide sequences are derived from at least about 30, 40, 50, 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% of the sequence corresponding to the polypeptide of interest.

[0015] The polypeptide of interest is suitably an antigen selected from a protein antigen, an antigen expressed by cancer cells, a particulate antigen, an alloantigen, an autoantigen or an allergen, or an immune complex. In certain embodiments, the polypeptide of interest is a disease- or condition-associated polypeptide such as but not limited to a polypeptide produced by a pathogenic organism or a cancer. Examples of pathogenic organisms include, but are not restricted to, yeast, viruses, bacteria,

helminths, protozoans and mycoplasmas. Examples of cancers include, but are not restricted to, melanoma, lung cancer, breast cancer, cervical cancer, prostate cancer, colon cancer, pancreatic cancer, stomach cancer, bladder cancer, kidney cancer, post transplant lymphoproliferative disease (PTLD), Hodgkin's Lymphoma and the like.

5 [0016] In another aspect, the invention provides antigen-presenting cells or their precursors which have been contacted with a set of peptides as broadly described above for a time and under conditions sufficient for the peptides or processed forms thereof to be presented by the antigen-presenting cells or by their precursors.

10 [0017] In a related aspect, the invention provides a process for producing antigen-presenting cells for modulating an immune response to a polypeptide of interest. The process generally comprises contacting antigen-presenting cells or their precursors with at least one set of peptides as broadly described above for a time and under conditions sufficient for the peptides or processed form thereof to be presented by the antigen-presenting cells or by their precursors. Suitably, when precursors are used, the precursors are cultured for a time and under conditions sufficient to differentiate antigen-presenting cells from the precursors.

15 [0018] In some embodiments, the or each set of peptides is contacted with substantially purified antigen-presenting cells or their precursors. In other embodiments, the or each set of peptides is contacted with a heterogeneous population of antigen-presenting cells or their precursors. In these embodiments, the heterogeneous pool of cells can be blood or peripheral blood mononuclear cells. Typically, the antigen-presenting cells or their precursors are selected from monocytes, macrophages, cells of myeloid lineage, B cells, dendritic cells or Langerhans cells. In still other embodiments, the or each set of peptides is contacted with an uncultured population of antigen-presenting cells or their precursors. The population can be homogenous or heterogeneous, illustrative examples of which include whole blood, fresh blood, or fractions thereof such as, but not limited to, peripheral blood mononuclear cells, buffy coat fractions of whole blood, packed red cells, irradiated blood, dendritic cells, monocytes, macrophages, neutrophils, lymphocytes, natural killer cells and natural killer T cells.

20 [0019] The antigen-presenting cells broadly described above are also useful for producing lymphocytes, including T lymphocytes and B lymphocytes, for modulating an immune response to a specified antigen or group of antigens. Accordingly, in yet another aspect, the invention provides a method for producing antigen-specific lymphocytes. The method comprises contacting a population of lymphocytes, or their precursors, with an antigen-presenting cell as broadly described above for a time and under conditions sufficient to produce the antigen-specific lymphocytes that modulate an immune response to at least one polypeptide from which the overlapping peptides were derived.

30 [0020] In yet another aspect, the invention contemplates a composition comprising at least one set of peptides, or the antigen-presenting cells, or the lymphocytes, as broadly described above, and a



pharmaceutically acceptable carrier and/or diluent. In certain embodiments, the composition may further comprise an adjuvant or compounds that stabilise the peptides or antigens against degradation by host enzymes.

5 [0021] In yet another aspect, the invention embraces a method for modulating an immune response to a polypeptide of interest, comprising administering to a patient in need of such treatment at least one set of peptides, or the antigen-presenting cells, or the lymphocytes, or the composition as broadly described above for a time and under conditions sufficient to modulate the immune response.

10 [0022] In a related aspect, the invention encompasses a method for treatment and/or prophylaxis of a disease or condition associated with the presence of a polypeptide of interest, comprising administering to a patient in need of such treatment or prophylaxis an effective amount of at least one set of peptides, or the antigen-presenting cells, or the lymphocytes, or the composition as broadly described above. In some embodiments, peptides or antigen-presenting cells or the lymphocytes are administered systemically, typically by injection.

15 [0023] In still yet another aspect, the invention contemplates the use of at least one set of peptides, or of the antigen-presenting cells, or of the lymphocytes, as broadly described above, in the preparation of a medicament for modulating an immune response to a polypeptide of interest or for treating or preventing a disease or condition associated with the presence of a polypeptide of interest.

20 [0024] The present invention also discloses the discovery that it is not necessary to culture a population of antigen-presenting cells or their precursors to expand that population prior to contacting it with a target antigen so that the contacted population is useful for modulating an immune response to the target antigen in a suitable recipient. Instead, the present inventors have unexpectedly discovered that uncultured antigen-presenting cells or their precursors, when contacted with an antigen that corresponds to a target antigen, are sufficient to modulate an immune response to the target antigen. The use of uncultured antigen-presenting cells or their precursors circumvents the need for  
25 expensive culturing and cell processing facilities and, in certain desirable embodiments, provides much faster vaccination regimens, as compared to current protocols. Additionally, the present inventors have discovered that it is not necessary to incubate the uncultured antigen-presenting cells under conditions that lead to their activation, in order to effectively modulate the immune response to the target antigen, which further reduces the number of process steps and manipulations.

30 [0025] Accordingly, in another aspect, the present invention features a composition of matter for modulating an immune response in a subject to a target antigen, the composition comprising uncultured antigen-presenting cells or their precursors, which have not been subjected to activating conditions, and which have been contacted with an antigen corresponding to the target antigen for a time (e.g., from about 1 minute to about 5 days) and under conditions sufficient to express a processed  
35 or modified form of the antigen for presentation to the subject's immune system (e.g., T lymphocytes).

Illustrative examples of uncultured cells include whole blood, fresh blood, or fractions thereof such as but not limited to peripheral blood mononuclear cells, buffy coat fractions of whole blood, packed red cells, irradiated blood, dendritic cells, monocytes, macrophages, neutrophils, lymphocytes, natural killer cells and natural killer T cells.

- 5 [0026] The antigen corresponding to the target antigen can be of any type including, for example, nucleic acids, peptides, hormones, whole protein antigens, cellular material (e.g., live or inactivated cancer cells), particulate matter such as, but not limited to, cell debris, apoptotic cells, lipid aggregates such as liposomes, membranous vehicles, microspheres, heat aggregated proteins, virosomes, virus-like particles and whole organisms including, for example, bacteria, mycobacteria,
- 10 viruses, fungi, protozoa or parts thereof. In some embodiments, the antigen is selected from a proteinaceous molecule or a nucleic acid molecule. In some embodiments, the uncultured cells are contacted with at two or more antigens. In illustrative examples of this type, the antigens are in the form of overlapping or non-overlapping peptides or one or more polynucleotides from which the peptides are expressible.
- 15 [0027] In a related aspect, the invention extends to the use of uncultured antigen-presenting cells or their precursors in the preparation of a medicament for the treatment of a disease or condition in a subject, which disease or condition is associated with the presence or aberrant expression of a target antigen, wherein the antigen-presenting cells or their precursors have not been subjected to activating conditions but have been contacted with an antigen that corresponds to the target antigen for a time
- 20 and under conditions sufficient to express a processed or modified form of the antigen for presentation to the subject's immune system.

### BRIEF DESCRIPTION OF THE DRAWINGS

[0028] Figure 1 is a schematic representation of an *in vivo* CTL killing assay performed at weeks 10, 15 and 20.

5 [0029] Figure 2 is a graphical representation showing *in vivo* CTL killing of SIVgag overlapping peptide-pulsed cells. Two weeks after the FPV-boost (week 10), 3 equal PBMC populations were labelled with SNARF (2.5 $\mu$ M) or CFSE (2.5  $\mu$ M or 0.25  $\mu$ M) and were pulsed with SIVpol, nef or gag overlapping peptide pools (OPAL), respectively. Blood sampled at 5 min, and at 4 and 16 h post-OPAL infusion was RBC-lysed and 10<sup>6</sup> lymphocyte events were acquired by flow cytometry. At 5 min, all 3 populations of labelled PBMC are of relatively equal numbers. By 4 and 16 hours,  
10 2xDNA/FPV-immunised monkey H20 displayed 27.3% and 76.0% clearance of SIVgag-pulsed PBMC with respect to SIVnef-pulsed PBMC, respectively, whereas no SIVgag-specific killing was observed in control-immunised monkey E20. Note that less events were collected at 4 h than 16 h.

[0030] Figure 3 is a graphical representation showing vigorous killing of SIVgag- and SIVpol-pulsed PBMC following SHIV challenge. Two weeks after SHIV challenge (week 20), equal PBMC  
15 populations were labelled with SNARF (5 $\mu$ M) or CFSE (6 $\mu$ M or 2.5  $\mu$ M) and were pulsed with SIVpol, no peptide, or SIVgag overlapping peptide pools (OPAL), respectively. 10<sup>6</sup> RBC-lysed lymphocyte events were acquired by flow cytometry. 2xDNA/FPV-immunised monkeys H20 and H21, Displayed 92.3% and 98.3% killing of SIVgag-pulsed PBMC. These animals received 2 separate infusions of SIVpol- pulsed PBMC, furthermore displaying >99% SIVpol-specific killing. Previously  
20 CFSE-labelled PBMC were accounted for by flow cytometric analysis of 10<sup>6</sup> lymphocytes immediately prior to OPAL-infusion (not shown).

[0031] Figure 4 is a photographic representation showing a boost in T-cell immunogenicity 1 week following OPAL-infusion analysed by IFN $\gamma$  ELISpot. A boost in SIVgag and pol peptide pool responses is evident in 2xDNA/FPV-immunised monkey H21, where as a primed response to SIVpol  
25 peptide pool is detected in control-immunised monkey E20 (week 10 shown above).

[0032] Figure 5 is a graphical representation depicting IFN $\gamma$  ELISpot analysis 1 week following OPAL infusion at week 10. A boost in T-cell immunogenicity to SIVgag, pol and nef overlapping peptide pools by OPAL infusion at week 10 was analysed 1 week later by ELISpot. Increased responses to SIVgag were detected in all four 2xDNA/FPV-immunised animals. Increased SIVpol  
30 responses were present in the 2xDNA/FPV-immunised monkeys, H20 and H21 (monkeys B00 and H8 did not receive any pol-pulsed PBMC), and in one control-immunised monkey, E20. No responses to SIVnef were primed in any animals. \*IFN $\gamma$  spots in monkeys E20 (prior to OPAL infusion) and B00 (post-OPAL infusion) were excluded due to ELISpot developmental problems.

[0033] Figure 6 is a graphical representation showing INF $\gamma$  ELISpot analysis 1 week following OPAL infusion at week 15. A boost in T-cell immunogenicity to SIVgag, pol, nef and HIV-1env overlapping peptide pools by OPAL infusion at week 15 was analysed 1 week later by INF $\gamma$  ELISpot. Increased responses to SIVgag were detected in all four 2xDNA/FPV-immunised animals. SIVpol responses were marginally increased (or primed) in monkeys, E22, B00, H20 and H21. Increased responses to WI SIV were evident in all animals, whereas no responses were detected for SIVnef or HIV-env in any animals.

[0034] Figure 7 is a graphical representation depicting mean INF $\gamma$  ELISpot of immunogenicity of OPAL infusion. Mean INF $\gamma$  ELISpot responses to (A) SIVgag and (B) SIVpol overlapping peptide pool of control- and 2xDNA/FPV-immunised animals receiving OPAL infusions (bold) were compared to animals receiving equivalent immunisations but no OPAL infusions, before and after the OPAL infusions given at weeks 10 and 15 following the immunisation. For the comparison of SIVpol-specific responses, 2xDNA/FPV-immunised animals were grouped based on receiving either 1 (B00 and H8) or 2 (H20 and H21) doses of pol-OPAL infusions.

[0035] Figure 8 is a graphical representation showing the outcome of SHIV intrarectal challenge. At week 18 all control- and 2xDNA/FPV-immunised macaques were challenged intrarectally with SHIV<sub>nm229</sub> and were assessed for plasma SHIV RNA viral load and CD4<sup>+</sup> T cell count over the course of the infection. Recipients of OPAL infusion were compared to their respective immunised non-OPAL recipients. Group comparisons indicate mean  $\pm$  SE. 2xDNA/FPV-immunised macaques receiving OPAL infusions were further grouped based on receiving either 1 or 2 separate doses of pol-pulsed PBMC (B00 & H8, and H20 & H21, respectively).

[0036] Figure 9 is a graphical representation depicting induction of CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses to SHIV antigens in monkeys infected with SHIV utilising administration of whole blood pulsed with overlapping 15mer peptides encompassing the open reading frames of the entire SHIV genome. The whole blood pulsed peptides were administered at weeks 0, 4 and 8 (arrows) and a boost in T cell immunogenicity of both CD4<sup>+</sup> and CD8<sup>+</sup> T cells measured by IFN $\gamma$  production to SHIV antigens gag, pol, env and rev-tat-vpu-nef detected by ICS is seen following each time point. \*Pre-OPAL T cells responses measured 1 week prior to 1<sup>st</sup> OPAL (week -1).

[0037] Figure 10 is a graphical representation depicting *de novo* induction of CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses to HCV in monkeys utilising administration of whole blood pulsed with overlapping 18mer peptides encompassing the open reading frames of the entire HCV type-1a H77 genome. The whole blood pulsed peptides were administered at weeks 0, 4 and 8 (arrows) in two separate pools (peptides: 1-116, and; 117-441). Induction and boosting of T cell immunogenicity of both CD4<sup>+</sup> and CD8<sup>+</sup> T cells measured by IFN $\gamma$  production to HCV antigens detected by ICS is seen following each time point. \*Pre-OPAL T cells responses measured 1 week prior to 1<sup>st</sup> OPAL (week -1).



[0038] Figure 11 is a graphical representation showing *de novo* induction of CD4+ and CD8+ T cell responses to peptides representative of drug-resistant mutations in HIV-1 described in HIV-1 infected humans, in monkeys utilising administration of whole blood pulsed with 17mer peptides encompassing known sites of reverse transcriptase or protease resistance mutations. The whole blood pulsed peptides were administered at weeks 0, 4 and 8 (arrows). Induction and boosting of T cell immunogenicity of both CD4+ and CD8+ T cells measured by IFN $\gamma$  production to HIV-1 drug-resistant mutation peptides detected by ICS is seen following each time point. \*Pre-OPAL T cells responses measured 1 week prior to 1<sup>st</sup> OPAL (week -1).

[0039] Figure 12 is a diagrammatic representation showing one embodiment of a pool of single peptides corresponding to drug-resistant mutations in the reverse transcriptase region or the protease region of wild-type HIV-1 described in HIV-1 humans (Mimotopes, Melbourne). 17mer peptides were designed spanning the sites of common known mutations to incorporate the resistant mutation at the 9<sup>th</sup> amino acid residue (bold) on each 17mer peptide, such that every 9mer epitope (the most common length of CD8+ T cell epitopes) as a result of proteolytic cleaving *ex vivo* would encompass the mutation.

## DETAILED DESCRIPTION OF THE INVENTION

### 1. Definitions

[0040] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are described. For the purposes of the present invention, the following terms are defined below.

[0041] The articles "*a*" and "*an*" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[0042] The term "*about*" is used herein to refer to conditions (e.g., amounts, concentrations, time etc) that vary by as much as 30%, preferably by as much as 20%, and more preferably by as much as 10% to a specified condition.

[0043] The term "*activating conditions*" refers to treatment conditions that lead to the expression of each of CD2, CD83, CD14, MHC class I, MHC class II and TNF- $\alpha$  at a level or functional activity that results from an activating treatment condition selected from: incubating the antigen-presenting cells or their precursors in the presence of an agent selected from cytokines (e.g., IL-4, GM-CSF or a type I interferon), chemokines, mitogens, lipopolysaccharide, or agents that induce interferon synthesis in the antigen-presenting cells or their precursors; or exposing the antigen-presenting cells or their precursors to physical stress. However, it shall be understood that the term "*activating conditions*" excludes treatment conditions that result in negligible activation of the cells, e.g., when less than about 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.2% or 0.1% of the cells are activated, or when each of CD2, CD83, CD14, MHC class I, MHC class II and TNF- $\alpha$  is expressed at a level or functional activity that is at least about 30%, 40%, 50%, 60%, 70%, 80% or 90%, or even at least about 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900% or 1000% higher, or at least about 30%, 40%, 50%, 60%, 70%, 80%, 90%, 92%, 94%, 96%, 97%, 98% or 99%, or even an at least about 99.5%, 99.9%, 99.95%, 99.99%, 99.995% or 99.999% lower than its level or functional activity in antigen-presenting cells or their precursors subjected to an activating treatment condition mentioned above.

[0044] By "*antigen*" is meant all, or part of, a protein, peptide, or other molecule or macromolecule capable of eliciting an immune response in a vertebrate animal, preferably a mammal. Such antigens are also reactive with antibodies from animals immunised with said protein, peptide, or other molecule or macromolecule.

[0045] By "*antigen-binding molecule*" is meant a molecule that has binding affinity for a target antigen. It will be understood that this term extends to immunoglobulins, immunoglobulin fragments and non-immunoglobulin derived protein frameworks that exhibit antigen-binding activity.

5 [0046] By "*autologous*" is meant something (e.g., cells, tissues etc) derived from the same organism.

[0047] The term "*allogeneic*" as used herein refers to cells, tissues, organisms etc that are of different genetic constitution.

10 [0048] Throughout this specification, unless the context requires otherwise, the words "*comprise*", "*comprises*" and "*comprising*" will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements.

15 [0049] By "*corresponds to*" or "*corresponding to*" is meant a polynucleotide (a) having a nucleotide sequence that is substantially identical or complementary to all or a portion of a reference polynucleotide sequence or (b) encoding an amino acid sequence identical to an amino acid sequence in a peptide or protein. This phrase also includes within its scope a peptide or polypeptide having an amino acid sequence that is substantially identical or similar to a sequence of amino acids in a reference peptide or protein.

20 [0050] As used herein, the terms "*culturing*", "*culture*" and the like refer to the set of procedures used *in vitro* where a population of cells (or a single cell) is incubated under conditions which have been shown to support the growth or maintenance of the cells *in vitro*. The art recognises a wide number of formats, media, temperature ranges, gas concentrations etc. which need to be defined in a culture system. The parameters will vary based on the format selected and the specific needs of the individual who practices the methods herein disclosed. However, it is recognised that the determination of culture parameters is routine in nature.

25 [0051] By "*effective amount*", in the context of modulating an immune response or treating or preventing a disease or condition, is meant the administration of that amount of composition to an individual in need thereof, either in a single dose or as part of a series, that is effective for that modulation, treatment or prevention. The effective amount will vary depending upon the health and physical condition of the individual to be treated, the taxonomic group of individual to be treated, the formulation of the composition, the assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

30 [0052] By "*expression vector*" is meant any autonomous genetic element capable of directing the synthesis of a protein encoded by the vector. Such expression vectors are known by practitioners in the art.

[0053] The term "*gene*" as used herein refers to any and all discrete coding regions of the cell's genome, as well as associated non-coding and regulatory regions. The gene is also intended to mean the open reading frame encoding specific polypeptides, introns, and adjacent 5' and 3' non-coding nucleotide sequences involved in the regulation of expression. In this regard, the gene may further  
5 comprise control signals such as promoters, enhancers, termination and/or polyadenylation signals that are naturally associated with a given gene, or heterologous control signals. The DNA sequences may be cDNA or genomic DNA or a fragment thereof. The gene may be introduced into an appropriate vector for extrachromosomal maintenance or for integration into the host.

[0054] A compound or composition is "*immunogenic*" if it is capable of either: a) generating an  
10 immune response against an antigen (e.g., a tumour antigen) in a naive individual; or b) reconstituting, boosting, or maintaining an immune response in an individual beyond what would occur if the compound or composition was not administered. A compound or composition is immunogenic if it is capable of attaining either of these criteria when administered in single or multiple doses.

[0055] Reference herein to "*immuno-interactive*" includes reference to any interaction, reaction,  
15 or other form of association between molecules and in particular where one of the molecules is, or mimics, a component of the immune system.

[0056] By "*isolated*" is meant material that is substantially or essentially free from components that normally accompany it in its native state.

[0057] By "*modulating*" is meant increasing or decreasing, either directly or indirectly, the  
20 immune response of an individual. In certain embodiments, "modulation" or "modulating" means that a desired/selected response is more efficient (e.g., at least 10%, 20%, 30%, 40%, 50%, 60% or more), more rapid (e.g., at least 10%, 20%, 30%, 40%, 50%, 60% or more), greater in magnitude (e.g., at least 10%, 20%, 30%, 40%, 50%, 60% or more), and/or more easily induced (e.g., at least 10%, 20%, 30%, 40%, 50%, 60% or more) than in the absence of an antigen or than if the antigen had been used  
25 alone.

[0058] The term "*operably connected*" or "*operably linked*" as used herein means placing a structural gene under the regulatory control of a promoter, which then controls the transcription and optionally translation of the gene. In the construction of heterologous promoter/structural gene combinations, it is generally preferred to position the genetic sequence or promoter at a distance from  
30 the gene transcription start site that is approximately the same as the distance between that genetic sequence or promoter and the gene it controls in its natural setting; i.e. the gene from which the genetic sequence or promoter is derived. As is known in the art, some variation in this distance can be accommodated without loss of function. Similarly, the preferred positioning of a regulatory sequence element with respect to a heterologous gene to be placed under its control is defined by the positioning  
35 of the element in its natural setting; i.e. the genes from which it is derived.



[0059] The terms "*patient*," "*subject*" and "*individual*" are used interchangeably herein to refer to any subject, particularly a vertebrate subject, and even more particularly a mammalian subject, for whom therapy or prophylaxis is desired. However, it will be understood that these terms do not imply that symptoms are present. Suitable vertebrate animals that fall within the scope of the invention include, but are not restricted to, primates, livestock animals (e.g., sheep, cows, horses, donkeys, pigs), laboratory test animals (e.g., rabbits, mice, rats, guinea pigs, hamsters), companion animals (e.g., cats, dogs) and captive wild animals (e.g., foxes, deer, dingoes, reptiles, avians, fish).

[0060] By "*pharmaceutically-acceptable carrier*" is meant a solid or liquid filler, diluent or encapsulating substance that may be safely used in topical or systemic administration.

10 [0061] The term "*polynucleotide*" or "*nucleic acid*" as used herein designates mRNA, RNA, cRNA, cDNA or DNA. The term typically refers to oligonucleotides greater than 30 nucleotides in length.

[0062] "*Polypeptide*", "*peptide*" and "*protein*" are used interchangeably herein to refer to a polymer of amino acid residues and to variants and synthetic analogues of the same. Thus, these terms apply to amino acid polymers in which one or more amino acid residues is a synthetic non-naturally occurring amino acid, such as a chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally-occurring amino acid polymers.

[0063] Reference herein to a "*promoter*" is to be taken in its broadest context and includes the transcriptional regulatory sequences of a classical genomic gene, including the TATA box which is required for accurate transcription initiation, with or without a CCAAT box sequence and additional regulatory elements (i.e. upstream activating sequences, enhancers and silencers) which alter gene expression in response to developmental and/or environmental stimuli, or in a tissue-specific or cell-type-specific manner. A promoter is usually, but not necessarily, positioned upstream or 5', of a structural gene, the expression of which it regulates. Furthermore, the regulatory elements comprising a promoter are usually positioned within 2 kb of the start site of transcription of the gene. Preferred promoters according to the invention may contain additional copies of one or more specific regulatory elements to further enhance expression in a cell, and/or to alter the timing of expression of a structural gene to which it is operably connected.

30 [0064] The term "*purified peptide*" means that the peptide is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the peptide is derived, or substantially free from chemical precursors or other chemicals when chemically synthesised. "Substantially free" means that a preparation of a peptide of the invention is at least 10% pure. In certain embodiments, the preparation of peptide has less than about 30%, 25%, 20%, 15%, 10% and desirably 5% (by dry weight), of non-peptide protein (also referred to herein as a

“contaminating protein”), or of chemical precursors or non-peptide chemicals. The invention includes isolated or purified preparations of at least 0.01, 0.1, 1.0, and 10 milligrams in dry weight.

5 [0065] The term “*recombinant polynucleotide*” as used herein refers to a polynucleotide formed *in vitro* by the manipulation of nucleic acid into a form not normally found in nature. For example, the recombinant polynucleotide may be in the form of an expression vector. Generally, such expression vectors include transcriptional and translational regulatory nucleic acid operably linked to the nucleotide sequence.

[0066] By “*recombinant polypeptide*” is meant a polypeptide made using recombinant techniques, i.e., through the expression of a recombinant polynucleotide.

10 [0067] By “*reporter molecule*” as used in the present specification is meant a molecule that, by its chemical nature, provides an analytically identifiable signal that allows the detection of a complex comprising an antigen-binding molecule and its target antigen. The term “reporter molecule” also extends to use of cell agglutination or inhibition of agglutination such as red blood cells on latex beads, and the like.

15 [0068] The term “*sequence identity*” as used herein refers to the extent that sequences are identical on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a “*percentage of sequence identity*” is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, I) or the identical amino acid residue (e.g., Ala, Pro, Ser,  
20 Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity. For the purposes of the present invention, “*sequence identity*” will be understood to mean the “match percentage” calculated by the DNASIS  
25 computer program (Version 2.5 for windows; available from Hitachi Software engineering Co., Ltd., South San Francisco, California, USA) using standard defaults as used in the reference manual accompanying the software.

[0069] “*Similarity*” refers to the percentage number of amino acids that are identical or constitute conservative substitutions as defined in Table B *infra*. Similarity may be determined using sequence  
30 comparison programs such as GAP (Deveraux *et al.* 1984, *Nucleic Acids Research* 12, 387-395). In this way, sequences of a similar or substantially different length to those cited herein might be compared by insertion of gaps into the alignment, such gaps being determined, for example, by the comparison algorithm used by GAP.

[0070] Terms used to describe sequence relationships between two or more polynucleotides or  
35 polypeptides include “reference sequence”, “comparison window”, “sequence identity”, “percentage

of sequence identity" and "substantial identity". A "reference sequence" is at least 12 but frequently 15 to 18 and often at least 25 monomer units, inclusive of nucleotides and amino acid residues, in length. Because two polynucleotides may each comprise (1) a sequence (i.e., only a portion of the complete polynucleotide sequence) that is similar between the two polynucleotides, and (2) a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window" refers to a conceptual segment of at least 6 contiguous positions, usually about 50 to about 100, more usually about 100 to about 150 in which a sequence is compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. The comparison window may comprise additions or deletions (i.e., gaps) of about 20% or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by computerised implementations of algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive Madison, WI, USA) or by inspection and the best alignment (i.e., resulting in the highest percentage homology over the comparison window) generated by any of the various methods selected. Reference also may be made to the BLAST family of programs as for example disclosed by Altschul *et al.*, 1997, *Nucl. Acids Res.* 25:3389. A detailed discussion of sequence analysis can be found in Unit 19.3 of Ausubel *et al.*, "Current Protocols in Molecular Biology", John Wiley & Sons Inc, 1994-1998, Chapter 15.

[0071] By "*substantially purified population*" and the like is meant that greater than about 80%, usually greater than about 90%, more usually greater than about 95%, typically greater than about 98%, and more typically greater than about 99% of the cells in the population are antigen-presenting cells of a chosen type.

[0072] The term "*uncultured*" as used herein refers to a population of cells (or a single cell), which have been removed from an animal and incubated or processed under conditions that do not result in the growth or expansion of the cells *in vitro*, or that result in negligible growth or expansion of the cells (e.g., an increase of less than about 50%, 40%, 30%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.2% or 0.1% in cell number as compared to the number of cells at the commencement of the incubation or processing). In certain desirable embodiments, the population of cells (or the single cell) is incubated or processed under conditions supporting the maintenance of the cells *in vitro*.

[0073] By "*vector*" is meant a nucleic acid molecule, preferably a DNA molecule derived, for example, from a plasmid, bacteriophage, or plant virus, into which a nucleic acid sequence may be inserted or cloned. A vector preferably contains one or more unique restriction sites and may be



capable of autonomous replication in a defined host cell including a target cell or tissue or a progenitor cell or tissue thereof, or be integrable with the genome of the defined host such that the cloned sequence is reproducible. Accordingly, the vector may be an autonomously replicating vector, i.e., a vector that exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, e.g., a linear or closed circular plasmid, an extrachromosomal element, a minichromosome, or an artificial chromosome. The vector may contain any means for assuring self-replication. Alternatively, the vector may be one which, when introduced into the host cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated. A vector system may comprise a single vector or plasmid, two or more vectors or plasmids, which together contain the total DNA to be introduced into the genome of the host cell, or a transposon. The choice of the vector will typically depend on the compatibility of the vector with the host cell into which the vector is to be introduced. The vector may also include a selection marker such as an antibiotic resistance gene that can be used for selection of suitable transformants.

## 2. *Immunomodulating sets of overlapping peptides*

[0074] The present invention is predicated in part on the discovery that antigen-presenting cells contacted *ex vivo* with a set of overlapping peptides spanning a viral polypeptide antigen of interest (also referred to herein as Overlapping Peptide-pulsed Autologous cells, OPAL) are effective in producing a strong immunogenic response in an outbred population, without prior knowledge of the epitopes of the antigen. Since antigen-presenting cells form a significant part of the circulatory system, it is proposed that systemic delivery of the overlapping peptides *per se* will produce a similar protective effect. Accordingly, the present invention broadly provides a set of peptides for modulating an immune response to a polypeptide of interest, wherein individual peptides comprise different portions of an amino acid sequence corresponding to the polypeptide of interest and display partial sequence identity or similarity to at least one other peptide of the set.

[0075] The partial sequence identity or similarity is typically contained at one or both ends of an individual peptide. In one embodiment, there are at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 40, 50 contiguous amino acid residues at one or both ends of an individual peptide, whose sequence is identical or similar to an amino acid sequence contained within at least one other of the peptides. In an alternate embodiment, there are less than 500, 100, 50, 40, 30 contiguous amino acid residues at one or both ends of an individual peptide, whose sequence is identical or similar to an amino acid sequence contained within at least one other of the peptides. Such 'sequence overlap' is advantageous to prevent or otherwise reduce the loss of any potential epitopes contained within a polypeptide of interest. In specific examples disclosed herein, the sequence overlap is 11 amino acid residues.



[0076] Typically, when peptides have partial sequence similarity, their sequences will usually differ by one or more conserved and/or non-conserved amino acid substitutions. Exemplary conservative substitutions are listed in the following table.

TABLE A

Original Residue	Exemplary Substitutions	Original Residue	Exemplary Substitutions
Ala	Ser	Leu	Ile, Val
Arg	Lys	Lys	Arg, Gln, Glu
Asn	Gln, His	Met	Leu, Ile,
Asp	Glu	Phe	Met, Leu, Tyr
Cys	Ser	Ser	Thr
Gln	Asn	Thr	Ser
Glu	Asp	Trp	Tyr
Gly	Pro	Tyr	Trp, Phe
His	Asn, Gln	Val	Ile, Leu
Ile	Leu, Val		

5

[0077] Conserved or non-conserved substitutions may correspond to polymorphisms in a polypeptide of interest. Polymorphic polypeptides are expressed by various pathogenic organisms and cancers. For example, the polymorphic polypeptides may be expressed by different viral strains or clades or by different cancers in distinct individuals. Thus, where polymorphic regions of a pathogen of interest are involved, it is generally desirable to use additional sets of peptides covering the variation in amino acid residue at the polymorphic site.

10

[0078] The peptides of the invention may be of any suitable size that can be utilised to elicit an immune response to a polypeptide of interest. A number of factors can influence the choice of peptide size. For example, the size of a peptide can be chosen such that it includes, or corresponds to the size of, CD4+ T cell epitopes, CD8+ T cell epitopes and/or B cell epitopes, and their processing requirements. Practitioners in the art will recognise that class I-restricted CD8+ T cell epitopes are typically between 8 and 10 amino acid residues in length and if placed next to unnatural flanking residues, such epitopes can generally require 2 to 3 natural flanking amino acid residues to ensure that they are efficiently processed and presented. Class II-restricted CD4+ T cell epitopes usually range between 12 and 25 amino acid residues in length and may not require natural flanking residues for efficient proteolytic processing although it is believed that natural flanking residues may play a role. Another important feature of class II-restricted epitopes is that they generally contain a core of 9-10 amino acid residues in the middle which bind specifically to class II MHC molecules with flanking sequences either side of this core stabilising binding by associating with conserved structures on either

15

20

side of class II MHC antigens in a sequence independent manner. Thus the functional region of class II-restricted epitopes is typically less than about 15 amino acid residues long. The size of linear B cell epitopes and the factors effecting their processing, like class II-restricted epitopes, are quite variable although such epitopes are frequently smaller in size than 15 amino acid residues. From the foregoing, it is advantageous, but not essential, that the size of the peptide is at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30 amino acid residues. Suitably, the size of the peptide is no more than about 500, 200, 100, 80, 60, 50, 40 amino acid residues. In one embodiment, the size of the peptide is large enough to minimise loss of T cell and/or B cell epitopes. In another embodiment, the size of the peptide is sufficient for presentation by an antigen-presenting cell of a T cell and/or a B cell epitope contained within the peptide. In one example of this embodiment, the size of the peptide is about 15 amino acid residues.

[0079] The polypeptide of interest is suitably a disease- or condition-associated antigen, which may be selected from endogenous antigens produced by an individual or exogenous antigens that are foreign to the individual. Suitable endogenous antigens include, but are not restricted to, self-antigens that are targets of autoimmune responses as well as cancer or tumour antigens. Illustrative examples of self antigens useful in the treatment or prevention of autoimmune disorders include, but not limited to, diabetes mellitus, arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis), multiple sclerosis, myasthenia gravis, systemic lupus erythematosus, autoimmune thyroiditis, dermatitis (including atopic dermatitis and eczematous dermatitis), psoriasis, Sjögren's Syndrome, including keratoconjunctivitis sicca secondary to Sjögren's Syndrome, alopecia areata, allergic responses due to arthropod bite reactions, Crohn's disease, ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, asthma, allergic asthma, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions, leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic encephalomyelitis, acute necrotizing haemorrhagic encephalopathy, idiopathic bilateral progressive sensorineural hearing loss, aplastic anaemia, pure red cell anaemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Stevens-Johnson syndrome, idiopathic sprue, lichen planus, Graves ophthalmopathy, sarcoidosis, primary biliary cirrhosis, uveitis posterior, and interstitial lung fibrosis. Other autoantigens include those derived from nucleosomes for the treatment of systemic lupus erythematosus (e.g., GenBank Accession No. D28394; Bruggen et al., 1996, *Ann. Med. Interne* (Paris), 147:485-489) and from the 44,000 Da peptide component of ocular tissue cross-reactive with *O. volvulus* antigen (McKeelnie et al., 1993, *Ann Trop. Med. Parasitol.* 87:649-652). Thus, illustrative autoantigens that can be used in the compositions and methods of the present invention include, but are not limited to, at least a portion of a lupus autoantigen, Smith, Ro, La, U1-RNP, fibrillin (scleroderma), pancreatic  $\beta$  cell antigens, GAD65 (diabetes related), insulin, myelin basic protein, myelin proteolipid protein, histones, PLP, collagen,

glucose-6-phosphate isomerase, citrullinated proteins and peptides, thyroid antigens, thyroglobulin, thyroid-stimulating hormone (TSH) receptor, various tRNA synthetases, components of the acetyl choline receptor (AchR), MOG, proteinase-3, myeloperoxidase, epidermal cadherin, acetyl choline receptor, platelet antigens, nucleic acids, nucleic acid:protein complexes, joint antigens, antigens of the nervous system, salivary gland proteins, skin antigens, kidney antigens, heart antigens, lung antigens, eye antigens, erythrocyte antigens, liver antigens and stomach antigens.

[0080] Non-limiting examples of cancer or tumour antigens include antigens from a cancer or tumour selected from ABL1 protooncogene, AIDS Related Cancers, Acoustic Neuroma, Acute Lymphocytic Leukaemia, Acute Myeloid Leukaemia, Adenocystic carcinoma, Adrenocortical Cancer, Agnogenic myeloid metaplasia, Alopecia, Alveolar soft-part sarcoma, Anal cancer, Angiosarcoma, Aplastic Anaemia, Astrocytoma, Ataxia-telangiectasia, Basal Cell Carcinoma (Skin), Bladder Cancer, Bone Cancers, Bowel cancer, Brain Stem Glioma, Brain and CNS Tumours, Breast Cancer, CNS tumours, Carcinoid Tumours, Cervical Cancer, Childhood Brain Tumours, Childhood Cancer, Childhood Leukaemia, Childhood Soft Tissue Sarcoma, Chondrosarcoma, Choriocarcinoma, Chronic Lymphocytic Leukaemia, Chronic Myeloid Leukaemia, Colorectal Cancers, Cutaneous T-Cell Lymphoma, Dermatofibrosarcoma-protuberans, Desmoplastic-Small-Round-Cell-Tumour, Ductal Carcinoma, Endocrine Cancers, Endometrial Cancer, Ependymoma, Esophageal Cancer, Ewing's Sarcoma, Extra-Hepatic Bile Duct Cancer, Eye Cancer, Eye: Melanoma, Retinoblastoma, Fallopian Tube cancer, Fanconi Anaemia, Fibrosarcoma, Gall Bladder Cancer, Gastric Cancer, Gastrointestinal Cancers, Gastrointestinal-Carcinoid-Tumour, Genitourinary Cancers, Germ Cell Tumours, Gestational-Trophoblastic-Disease, Glioma, Gynaecological Cancers, Haematological Malignancies, Hairy Cell Leukaemia, Head and Neck Cancer, Hepatocellular Cancer, Hereditary Breast Cancer, Histiocytosis, Hodgkin's Disease, Human Papillomavirus, Hydatidiform mole, Hypercalcemia, Hypopharynx Cancer, IntraOcular Melanoma, Islet cell cancer, Kaposi's sarcoma, Kidney Cancer, Langerhan's-Cell-Histiocytosis, Laryngeal Cancer, Leiomyosarcoma, Leukaemia, Li-Fraumeni Syndrome, Lip Cancer, Liposarcoma, Liver Cancer, Lung Cancer, Lymphedema, Lymphoma, Hodgkin's Lymphoma, Non-Hodgkin's Lymphoma, Male Breast Cancer, Malignant-Rhabdoid-Tumour-of-Kidney, Medulloblastoma, Melanoma, Merkel Cell Cancer, Mesothelioma, Metastatic Cancer, Mouth Cancer, Multiple Endocrine Neoplasia, Mycosis Fungoides, Myelodysplastic Syndromes, Myeloma, Myeloproliferative Disorders, Nasal Cancer, Nasopharyngeal Cancer, Nephroblastoma, Neuroblastoma, Neurofibromatosis, Nijmegen Breakage Syndrome, Non-Melanoma Skin Cancer, Non-Small-Cell-Lung-Cancer-(NSCLC), Ocular Cancers, Oesophageal Cancer, Oral cavity Cancer, Oropharynx Cancer, Osteosarcoma, Ostomy Ovarian Cancer, Pancreas Cancer, Paranasal Cancer, Parathyroid Cancer, Parotid Gland Cancer, Penile Cancer, Peripheral-Neuroectodermal-Tumours, Pituitary Cancer, Polycythemia vera, Prostate Cancer, Rare-cancers-and-associated-disorders, Renal Cell Carcinoma, Retinoblastoma, Rhabdomyosarcoma, Rothmund-



Thomson Syndrome, Salivary Gland Cancer, Sarcoma, Schwannoma, Sezary syndrome, Skin Cancer, Small Cell Lung Cancer (SCLC), Small Intestine Cancer, Soft Tissue Sarcoma, Spinal Cord Tumours, Squamous-Cell-Carcinoma-(skin), Stomach Cancer, Synovial sarcoma, Testicular Cancer, Thymus Cancer, Thyroid Cancer, Transitional-Cell-Cancer-(bladder), Transitional-Cell-Cancer-(renal-pelvis/-ureter), Trophoblastic Cancer, Urethral Cancer, Urinary System Cancer, Uroplakins, Uterine sarcoma, Uterus Cancer, Vaginal Cancer, Vulva Cancer, Waldenstrom's-Macroglobulinemia, Wilms' Tumour. In certain embodiments, the cancer or tumour relates to melanoma. Illustrative examples of melanoma-related antigens include melanocyte differentiation antigen (e.g., gp100, MART, TRP-1, Tyros, TRP2, MC1R, MUC1F, MUC1R or a combination thereof) and melanoma-specific antigens (e.g., BAGE, GAGE-1, gp100In4, MAGE-1 (e.g., GenBank Accession No. X54156 and AA494311), MAGE-3, MAGE4, PRAME, TRP2IN2, NYNSO1a, NYNSO1b, LAGE1, p97 melanoma antigen (e.g., GenBank Accession No. M12154) or a combination thereof). Other tumour-specific antigens include the Ras peptide and p53 peptide associated with advanced cancers, MUC1-KLH antigen associated with breast carcinoma (e.g., GenBank Accession No. J03651), CEA (carcinoembryonic antigen) associated with colorectal cancer (e.g., GenBank Accession No. X98311), gp100 (e.g., GenBank Accession No. S73003) and the PSA antigen with prostate cancer (e.g., GenBank Accession No. X14810). The p53 gene sequence is known (See e.g., Harris *et al.*, 1986 *Mol. Cell. Biol.* 6:4650-4656) and is deposited with GenBank under Accession No. M14694.

[0081] Foreign antigens are suitably selected from transplantation antigens, allergens as well as antigens from pathogenic organisms. Transplantation antigens can be derived from donor cells or tissues from e.g., heart, lung, liver, pancreas, kidney, neural graft components, or from the donor antigen-presenting cells bearing MHC loaded with self antigen in the absence of exogenous antigen.

[0082] Non-limiting examples of allergens include Fel d 1 (i.e., the feline skin and salivary gland allergen of the domestic cat *Felis domesticus*, the amino acid sequence of which is disclosed International Publication WO 91/06571), Der p I, Der p II, Der fI or Der fII (i.e., the major protein allergens from the house dust mite dermatophagoides, the amino acid sequence of which is disclosed in International Publication WO 94/24281). Other allergens may be derived, for example from the following: grass, tree and weed (including ragweed) pollens; fungi and moulds; foods such as fish, shellfish, crab, lobster, peanuts, nuts, wheat gluten, eggs and milk; stinging insects such as bee, wasp, and hornet and the chironomidae (non-biting midges); other insects such as the housefly, fruitfly, sheep blow fly, screw worm fly, grain weevil, silkworm, honeybee, non-biting midge larvae, bee moth larvae, mealworm, cockroach and larvae of *Tenibrio molitor* beetle; spiders and mites, including the house dust mite; allergens found in the dander, urine, saliva, blood or other bodily fluid of mammals such as cat, dog, cow, pig, sheep, horse, rabbit, rat, guinea pig, mouse and gerbil; airborne particulates in general; latex; and protein detergent additives.



[0083] Exemplary pathogenic organisms include, but are not limited to, viruses, bacteria, fungi parasites, algae and protozoa and amoebae. Illustrative examples of viruses include viruses responsible for diseases including, but not limited to, measles, mumps, rubella, poliomyelitis, hepatitis A, B (e.g., GenBank Accession No. E02707), and C (e.g., GenBank Accession No. E06890), as well as other hepatitis viruses, influenza, adenovirus (e.g., types 4 and 7), rabies (e.g., GenBank Accession No. M34678), yellow fever, Epstein-Barr virus and other herpesviruses such as papillomavirus, Ebola virus, influenza virus, Japanese encephalitis (e.g., GenBank Accession No. E07883), dengue (e.g., GenBank Accession No. M24444), hantavirus, sendai virus, respiratory syncytial virus, orthomyxoviruses, vesicular stomatitis virus, visna virus, cytomegalovirus and human immunodeficiency virus (HIV) (e.g., GenBank Accession No. U18552). Any suitable antigen derived from such viruses are useful in the practice of the present invention. For example, illustrative retroviral antigens derived from HIV include, but are not limited to, antigens such as gene products of the *gag*, *pol*, and *env* genes, the Nef protein, reverse transcriptase, and other HIV components. Illustrative examples of hepatitis viral antigens include, but are not limited to, antigens such as the S, M, and L proteins of hepatitis B virus, the pre-S antigen of hepatitis B virus, and other hepatitis, e.g., hepatitis A, B, and C, viral components such as hepatitis C viral RNA. Illustrative examples of influenza viral antigens include; but are not limited to, antigens such as hemagglutinin and neuraminidase and other influenza viral components. Illustrative examples of measles viral antigens include, but are not limited to, antigens such as the measles virus fusion protein and other measles virus components. Illustrative examples of rubella viral antigens include, but are not limited to, antigens such as proteins E1 and E2 and other rubella virus components; rotaviral antigens such as VP7sc and other rotaviral components. Illustrative examples of cytomegaloviral antigens include, but are not limited to, antigens such as envelope glycoprotein B and other cytomegaloviral antigen components. Non-limiting examples of respiratory syncytial viral antigens include antigens such as the RSV fusion protein, the M2 protein and other respiratory syncytial viral antigen components. Illustrative examples of herpes simplex viral antigens include, but are not limited to, antigens such as immediate early proteins, glycoprotein D, and other herpes simplex viral antigen components. Non-limiting examples of varicella zoster viral antigens include antigens such as 9PI, gpII, and other varicella zoster viral antigen components. Non-limiting examples of Japanese encephalitis viral antigens include antigens such as proteins E, M-E, M-E-NS 1, NS 1, NS 1-NS2A, 80%E, and other Japanese encephalitis viral antigen components. Illustrative examples of rabies viral antigens include, but are not limited to, antigens such as rabies glycoprotein, rabies nucleoprotein and other rabies viral antigen components. Illustrative examples of papillomavirus antigens include, but are not limited to, the L1 and L2 capsid proteins as well as the E6/E7 antigens associated with cervical cancers, See Fundamental Virology, Second Edition, eds. Fields, B.N. and Knipe, D.M., 1991, Raven Press, New York, for additional examples of viral antigens.

[0084] Illustrative examples of fungi include *Acremonium* spp., *Aspergillus* spp., *Basidiobolus* spp., *Bipolaris* spp., *Blastomyces dermatidis*, *Candida* spp., *Cladophialophora carrionii*, *Coccidioides immitis*, *Conidiobolus* spp., *Cryptococcus* spp., *Curvularia* spp., *Epidermophyton* spp., *Exophiala jeanselmei*, *Exserohilum* spp., *Fonsecaea compacta*, *Fonsecaea pedrosoi*, *Fusarium* 5 *oxysporum*, *Fusarium solani*, *Geotrichum candidum*, *Histoplasma capsulatum* var. *capsulatum*, *Histoplasma capsulatum* var. *duboisii*, *Hortaea werneckii*, *Lacazia loboi*, *Lasiodiplodia theobromae*, *Leptosphaeria senegalensis*, *Madurella grisea*, *Madurella mycetomatis*, *Malassezia furfur*, *Microsporum* spp., *Neotestudina rosatii*, *Onychocola canadensis*, *Paracoccidioides brasiliensis*, *Phialophora verrucosa*, *Piedraia hortae*, *Piedra iahortae*, *Pityriasis versicolor*, *Pseudallesheria* 10 *boydii*, *Pyrenochaeta romeroi*, *Rhizopus arrhizus*, *Scopulariopsis brevicaulis*, *Scytalidium dimidiatum*, *Sporothrix schenckii*, *Trichophyton* spp., *Trichosporon* spp., Zygomycete fungi, *Absidia corymbifera*, *Rhizomucor pusillus* and *Rhizopus arrhizus*. Thus, illustrative fungal antigens that can be used in the compositions and methods of the present invention include, but are not limited to, candida fungal antigen components; histoplasma fungal antigens such as heat shock protein 60 (HSP60) and other 15 histoplasma fungal antigen components; cryptococcal fungal antigens such as capsular polysaccharides and other cryptococcal fungal antigen components; coccidioides fungal antigens such as spherule antigens and other coccidioides fungal antigen components; and tinea fungal antigens such as trichophytin and other coccidioides fungal antigen components.

[0085] Illustrative examples of bacteria include bacteria that are responsible for diseases 20 including, but not restricted to, diphtheria (e.g., *Corynebacterium diphtheria*), pertussis (e.g., *Bordetella pertussis*, GenBank Accession No. M35274), tetanus (e.g., *Clostridium tetani*, GenBank Accession No. M64353), tuberculosis (e.g., *Mycobacterium tuberculosis*), bacterial pneumonias (e.g., *Haemophilus influenzae*), cholera (e.g., *Vibrio cholerae*), anthrax (e.g., *Bacillus anthracis*), typhoid, plague, shigellosis (e.g., *Shigella dysenteriae*), botulism (e.g., *Clostridium botulinum*), 25 salmonellosis (e.g., GenBank Accession No. L03833), peptic ulcers (e.g., *Helicobacter pylori*), Legionnaire's Disease, Lyme disease (e.g., GenBank Accession No. U59487). Other pathogenic bacteria include *Escherichia coli*, *Clostridium perfringens*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogenes*. Thus, bacterial antigens which can be used in the compositions and methods of the invention include, but are not limited to: pertussis bacterial antigens such as 30 pertussis toxin, filamentous hemagglutinin, pertactin, F M2, FIM3, adenylate cyclase and other pertussis bacterial antigen components; diphtheria bacterial antigens such as diphtheria toxin or toxoid and other diphtheria bacterial antigen components; tetanus bacterial antigens such as tetanus toxin or toxoid and other tetanus bacterial antigen components, streptococcal bacterial antigens such as M proteins and other streptococcal bacterial antigen components; gram-negative bacilli bacterial 35 antigens such as lipopolysaccharides and other gram-negative bacterial antigen components; *Mycobacterium tuberculosis* bacterial antigens such as mycolic acid, heat shock protein 65 (HSP65),

the 30kDa major secreted protein, antigen 85A and other mycobacterial antigen components; *Helicobacter pylori* bacterial antigen components, pneumococcal bacterial antigens such as pneumolysin, pneumococcal capsular polysaccharides and other pneumococcal bacterial antigen components; *Haemophilus influenza* bacterial antigens such as capsular polysaccharides and other

5 *Haemophilus influenza* bacterial antigen components; anthrax bacterial antigens such as anthrax protective antigen and other anthrax bacterial antigen components; rickettsiae bacterial antigens such as rompA and other rickettsiae bacterial antigen component. Also included with the bacterial antigens described herein are any other bacterial, mycobacterial, mycoplasmal, rickettsial, or chlamydial antigens.

10 [0086] Illustrative examples of protozoa include protozoa that are responsible for diseases including, but not limited to, malaria (e.g., GenBank Accession No. X53832), hookworm, onchocerciasis (e.g., GenBank Accession No. M27807), schistosomiasis (e.g., GenBank Accession No. LOS 198), toxoplasmosis, trypanosomiasis, leishmaniasis, giardiasis (GenBank Accession No. M33641), amoebiasis, filariasis (e.g., GenBank Accession No. J03266), borreliosis, and trichinosis.

15 Thus, protozoal antigens which can be used in the compositions and methods of the invention include, but are not limited to: plasmodium falciparum antigens such as merozoite surface antigens, sporozoite surface antigens, circumsporozoite antigens, gametocyte/gamete surface antigens, blood-stage antigen pf 155/RESA and other plasmodial antigen components; toxoplasma antigens such as SAG-1, p30 and other toxoplasma antigen components; schistosomae antigens such as glutathione-

20 S-transferase, paramyosin, and other schistosomal antigen components; leishmania major and other leishmaniae antigens such as gp63, lipophosphoglycan and its associated protein and other leishmanial antigen components; and trypanosoma cruzi antigens such as the 75-77kDa antigen, the 56kDa antigen and other trypanosomal antigen components.

[0087] The present invention also contemplates toxin components as antigens. Illustrative

25 examples of toxins include, but are not restricted to, staphylococcal enterotoxins, toxic shock syndrome toxin; retroviral antigens (e.g., antigens derived from HIV), streptococcal antigens, staphylococcal enterotoxin-A (SEA), staphylococcal enterotoxin-B (SEB), staphylococcal enterotoxin<sub>1-3</sub> (SE<sub>1-3</sub>), staphylococcal enterotoxin-D (SED), staphylococcal enterotoxin-E (SEE) as well as toxins derived from mycoplasma, mycobacterium, and herpes viruses.

30 [0088] In one example of the present invention, the size of individual peptides is about 14 or 15 amino acid residues and the sequence overlap at one or both ends of an individual peptide is about 11 amino acid residues. However, it will be understood that other suitable peptide sizes and sequence overlap sizes are contemplated by the present invention, which can be readily ascertained by persons of skill in the art.



[0089] It is advantageous but not necessary to utilise the entire sequence of a polypeptide of interest for producing a set of overlapping peptides. Typically, at least 30%, 40%, 50%, 60%, 70%, 80% 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% of the sequence corresponding to a polypeptide of interest is used to produce the overlapping peptides of the invention. However, it will be understood that the more sequence information from a polypeptide of interest that is utilised to produce the overlapping peptides, the greater the outbred population coverage will be of the overlapping peptides as an immunogen. Suitably, no sequence information from the polypeptide of interest is excluded (e.g., because of an apparent lack of immunological epitopes, since more rare or subdominant epitopes may be inadvertently missed). If required, hypervariable sequences within a polypeptide of interest can be either excluded from the construction of an overlapping set of peptides, or additional sets of peptides covering the polymorphic regions can be constructed and administered. Peptide sequences may include additional sequences that are not derived from a polypeptide of interest. These additional sequences may have various functions, including improving solubility, stability or immunogenicity or facilitating purification. Typically, such additional sequences are contained at one or both ends of a respective peptide.

[0090] Persons of skill in the art will appreciate that when preparing a set of overlapping peptides according to the invention, it may be advantageous to use sequence information from a plurality of different polypeptides produced by a pathogenic organism or expressed in a cancer. Accordingly, in certain embodiments, at least 2, 3, 4, 5, 6, 7, 9, 10, 15, 20 other sets of peptides are used for the production of the immunomodulating compositions of the invention, wherein the sequences of a respective other set of peptides are derived from a distinct polypeptide of interest and wherein individual peptides of the respective other set display partial sequence identity or similarity to at least one other peptide of a corresponding set of peptides. It is advantageous in this respect to utilise as many polypeptides as possible from, or in relation to, a particular source in the construction of sets of overlapping peptides. Suitably, at least about 30%, 40%, 50%, 60%, 70%, 80% 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and desirably 100%, of the polypeptides expressed by the source is used in the construction of the corresponding sets of overlapping peptides. Exemplary viral polypeptides that can be used for such construction include, but are not restricted to, latent polypeptides, regulatory polypeptides or polypeptides expressed early during their replication cycle. Suitably, polypeptides from a protozoan, bacterium, mycoplasma, fungus or helminth include, but are not restricted to, secretory polypeptides, regulatory polypeptides and polypeptides expressed on the surface of these organisms. Polypeptides from a cancer or tumour, which can be used for the construction of overlapping peptide sets, are suitably cancer-specific polypeptides.

[0091] Representative overlapping peptide sets for modulating the immune response to simian immunodeficiency virus (SIV) and/or the chimeric SIV-HIV-1 (SHIV), both of which are known to be suitable models for the pathogenic HIV-1 virus in humans, can be based on one or more polypeptides



selected from SIV gag, pol, nef or SHIV env as for example presented in Tables 1 to 4. Illustrative overlapping peptide sets for modulating the immune response to HIV-1 can be based on one or more polypeptides selected from HIV Gag, Nef, Pol, Rev, Tat, Vif, Vpr and Vpu as for example set forth in Tables 5 to 12. An illustrative overlapping peptide set for modulating the immune response to HCV 1a can be based on the HCV 1a H77 polyprotein sequence as for example set forth in Table 13. An illustrative overlapping peptide set for modulating the immune response to HBV Genotype A can be based on all proteins expressed by this genotype and on some portions of proteins expressed from Genotypes B/C/D, which display significant variability from Genotype A sequence, as for example set forth in Table 14.

10 [0092] The overlapping peptide sets of the invention may be prepared by any suitable procedure known to those of skill in the art. For example, the peptide sets can be synthesised conveniently using solution synthesis or solid phase synthesis as described, for example, in Chapter 9 of Atherton and Shephard (1989, *Solid Phase Peptide Synthesis: A Practical Approach*. IRL Press, Oxford) and in Roberge *et al* (1995, *Science* 269: 202). Syntheses may employ, for example, either *t*-butyloxycarbonyl (*t*-Boc) or 9-fluorenylmethyloxycarbonyl (Fmoc) chemistries (see Chapter 9.1, of Coligan *et al.*, *CURRENT PROTOCOLS IN PROTEIN SCIENCE*, John Wiley & Sons, Inc. 1995-1997; Stewart and Young, 1984, *Solid Phase Peptide Synthesis*, 2nd ed. Pierce Chemical Co., Rockford, Ill; and Atherton and Shephard, *supra*). In specific embodiments, the individual peptides are solubilized in DMSO (e.g., 100% pure DMSO) at high concentration (1 mg peptide/10-30  $\mu$ L DMSO) so that large pools of peptides do not contain excessive amounts of DMSO when pulsed onto cells. In certain advantageous embodiments, one or more peptide sets of the invention, in soluble form, are placed into a single container for convenient administration (e.g. a blood tube or vial for ready re-infusion) to a subject and such containers are also contemplated by the present invention.

15 [0093] Alternatively, individual peptides may be prepared by a procedure including the steps of:  
25 (a) preparing a synthetic construct including a synthetic polynucleotide encoding an individual peptide of an overlapping set of peptides, wherein the synthetic polynucleotide is operably linked to a regulatory polynucleotide; (b) introducing the synthetic construct into a suitable host cell; (c) culturing the host cell to express the synthetic polynucleotide; and (d) isolating the individual peptide. The synthetic construct is preferably in the form of an expression vector. For example, the expression vector can be a self-replicating extra-chromosomal vector such as a plasmid, or a vector that integrates into a host genome. Typically, the regulatory polynucleotide includes, but is not limited to, promoter sequences, leader or signal sequences, ribosomal binding sites, transcriptional start and stop sequences, translational start and termination sequences, and enhancer or activator sequences. Constitutive or inducible promoters as known in the art are contemplated by the invention. The promoters may be either naturally occurring promoters, or hybrid promoters that combine elements of more than one promoter. The regulatory polynucleotide will generally be appropriate for the host cell

used for expression. Numerous types of appropriate expression vectors and suitable regulatory polynucleotides are known in the art for a variety of host cells. In certain embodiments, the expression vector contains a selectable marker gene to allow the selection of transformed host cells. Selection genes are well known in the art and will vary with the host cell used. In other embodiments, the expression vector also includes a nucleic acid sequence that codes for a fusion partner so that an individual peptide is expressed as a fusion polypeptide with the fusion partner. The main advantage of fusion partners is that they assist identification and/or purification of the fusion polypeptide. Exemplary fusion partners include, but are not limited to, glutathione-S-transferase (GST), Fc portion of human IgG, maltose binding protein (MBP) and hexahistidine (HIS<sub>6</sub>), which are particularly useful for isolation of the fusion polypeptide by affinity chromatography. For the purposes of fusion polypeptide purification by affinity chromatography, relevant matrices for affinity chromatography are glutathione-, amylose-, and nickel- or cobalt-conjugated resins respectively. Many such matrices are available in "kit" form, such as the QIAexpress™ system (Qiagen) useful with (HIS<sub>6</sub>) fusion partners and the Pharmacia GST purification system. In a preferred embodiment, the recombinant polynucleotide is expressed in the commercial vector pFLAG™. Advantageously, the fusion partners also have protease cleavage sites, such as for Factor X<sub>a</sub>, Thrombin and inteins (protein introns), which allow the relevant protease to partially digest the fusion polypeptide of the invention and thereby liberate the recombinant polypeptide of the invention therefrom. The liberated peptide can then be isolated from the fusion partner by subsequent chromatographic separation. Fusion partners according to the invention also include within their scope "epitope tags", which are usually short peptide sequences for which a specific antibody is available. Well known examples of epitope tags for which specific monoclonal antibodies are readily available include c-Myc, influenza virus, haemagglutinin and FLAG tags.

[0094] The step of introducing the synthetic construct into the host cell may be achieved using any suitable technique including transfection, and transformation, the choice of which will be dependent on the host cell employed. Such methods are well known to those of skill in the art. The peptides of the invention may be produced by culturing a host cell transformed with the synthetic construct. The conditions appropriate for protein expression will vary with the choice of expression vector and the host cell. This is easily ascertained by one skilled in the art through routine experimentation. Suitable host cells for expression may be prokaryotic or eukaryotic. One preferred host cell for expression of a polypeptide according to the invention is a bacterium. The bacterium used may be *Escherichia coli*. Alternatively, the host cell may be an insect cell such as, for example, SF9 cells that may be utilised with a baculovirus expression system.

[0095] The amino acids of the peptides can be any non-naturally occurring or any naturally occurring amino acid. Examples of unnatural amino acids and derivatives during peptide synthesis include but are not limited to, use of 4-amino butyric acid, 6-aminohexanoic acid, 4-amino-3-hydroxy-

5-phenylpentanoic acid, 4-amino-3-hydroxy-6-methylheptanoic acid, t-butylglycine, norleucine, norvaline, phenylglycine, ornithine, sarcosine, 2-thienyl alanine and/or D-isomers of amino acids. A list of unnatural amino acids contemplated by the present invention is shown in TABLE B.

TABLE B

Non-conventional amino acid	Non-conventional amino acid
$\alpha$ -aminobutyric acid	L-N-methylalanine
$\alpha$ -amino- $\alpha$ -methylbutyrate	L-N-methylarginine
aminocyclopropane-carboxylate	L-N-methylasparagine
aminoisobutyric acid	L-N-methylaspartic acid
aminonorbornyl-carboxylate	L-N-methylcysteine
cyclohexylalanine	L-N-methylglutamine
cyclopentylalanine	L-N-methylglutamic acid
L-N-methylisoleucine	L-N-methylhistidine
D-alanine	L-N-methylleucine
D-arginine	L-N-methyllysine
D-aspartic acid	L-N-methylmethionine
D-cysteine	L-N-methylnorleucine
D-glutamate	L-N-methylnorvaline
D-glutamic acid	L-N-methylornithine
D-histidine	L-N-methylphenylalanine
D-isoleucine	L-N-methylproline
D-leucine	L-N-methylserine
D-lysine	L-N-methylthreonine
D-methionine	L-N-methyltryptophan
D-ornithine	L-N-methyltyrosine
D-phenylalanine	L-N-methylvaline
D-proline	L-N-methylethylglycine
D-serine	L-N-methyl-t-butylglycine
D-threonine	L-norleucine
D-tryptophan	L-norvaline
D-tyrosine	$\alpha$ -methyl-aminoisobutyrate
D-valine	$\alpha$ -methyl- $\gamma$ -aminobutyrate
D- $\alpha$ -methylalanine	$\alpha$ -methylcyclohexylalanine
D- $\alpha$ -methylarginine	$\alpha$ -methylcyclopentylalanine
D- $\alpha$ -methylasparagine	$\alpha$ -methyl- $\alpha$ -naphthylalanine
D- $\alpha$ -methylaspartate	$\alpha$ -methylpenicillamine

Non-conventional amino acid	Non-conventional amino acid
D- $\alpha$ -methylcysteine	N-(4-aminobutyl)glycine
D- $\alpha$ -methylglutamine	N-(2-aminoethyl)glycine
D- $\alpha$ -methylhistidine	N-(3-aminopropyl)glycine
D- $\alpha$ -methylisoleucine	N-amino- $\alpha$ -methylbutyrate
D- $\alpha$ -methyllleucine	$\alpha$ -naphthylalanine
D- $\alpha$ -methyllysine	N-benzylglycine
D- $\alpha$ -methylmethionine	N-(2-carbamylethyl)glycine
D- $\alpha$ -methylornithine	N-(carbamylmethyl)glycine
D- $\alpha$ -methylphenylalanine	N-(2-carboxyethyl)glycine
D- $\alpha$ -methylproline	N-(carboxymethyl)glycine
D- $\alpha$ -methylserine	N-cyclobutylglycine
D- $\alpha$ -methylthreonine	N-cycloheptylglycine
D- $\alpha$ -methyltryptophan	N-cyclohexylglycine
D- $\alpha$ -methyltyrosine	N-cyclodecylglycine
L- $\alpha$ -methyllleucine	L- $\alpha$ -methyllysine
L- $\alpha$ -methylmethionine	L- $\alpha$ -methylnorleucine
L- $\alpha$ -methylnorvaline	L- $\alpha$ -methylornithine
L- $\alpha$ -methylphenylalanine	L- $\alpha$ -methylproline
L- $\alpha$ -methylserine	L- $\alpha$ -methylthreonine
L- $\alpha$ -methyltryptophan	L- $\alpha$ -methyltyrosine
L- $\alpha$ -methylvaline	L-N-methylhomophenylalanine
N-(N-(2,2-diphenylethyl carbamylmethyl)glycine	N-(N-(3,3-diphenylpropyl carbamylmethyl)glycine
1-carboxy-1-(2,2-diphenyl-ethyl amino)cyclopropane	

[0096] The invention also contemplates modifying the peptides of the invention using ordinary molecular biological techniques so as to alter their resistance to proteolytic degradation or to optimise solubility properties or to render them more suitable as an immunogenic agent.

### 5 3. *Antigen-presenting cell embodiments*

[0097] The present invention also discloses the discovery that antigen-presenting cells which have been contacted with overlapping peptide sets as described in Section 2 are potent modulators of immune responses and are especially useful for raising strong immunogenic responses that can prevent or ameliorate the symptoms of a disease or condition of interest. Accordingly, the invention provides a process for producing antigen-specific antigen-presenting cells, comprising contacting antigen-presenting cells or their precursors with one or more sets of peptides as broadly described above for a



time and under conditions sufficient for the peptides or processed forms thereof to be presented by the antigen-presenting cells or their precursors, and in the case of precursors, culturing the precursors for a time and under conditions sufficient to differentiate antigen-presenting cells from the precursors.

[0098] The present inventors have also found unexpectedly that, in contrast to current dogma, it is not necessary to culture or activate purified antigen-presenting cells to increase their number or efficiency before loading them with antigen for effective modulation of an immune response to the antigen in a recipient of those cells. Instead, the present inventors have discovered that an uncultured population of antigen-presenting cells or their precursors, which have not been subjected to activating conditions, when contacted with an antigen that corresponds to a target antigen of interest is sufficient to effectively modulate an immune response to the target antigen in a recipient of the contacted population. Accordingly, in another aspect, the present invention provides a process for producing antigen-specific antigen-presenting cells, comprising contacting an uncultured population of antigen-presenting cells or their precursors, which have not been subjected to activating conditions, with an antigen corresponding to the target antigen for a time and under conditions sufficient for the antigen-presenting cells or their precursors to express a processed or modified form of the antigen. Illustrative examples of the uncultured population of antigen-presenting cells or their precursors include whole blood, fresh blood, or fractions thereof such as but not limited to peripheral blood mononuclear cells (PMBC), buffy coat fractions of whole blood, packed red cells, irradiated blood, dendritic cells, monocytes, macrophages, neutrophils, lymphocytes, natural killer cells and natural killer T cells. In specific embodiments, the uncultured population of antigen-presenting cells is selected from freshly isolated blood or PMBC. In other embodiments, the uncultured population of antigen-presenting cells is a necrotic or apoptotic population. Thus, the uncultured population of cells may be contacted with antigen and subsequently subjected to necrotic conditions, which lead to irreversible trauma to cells (e.g., osmotic shock or exposure to chemical poison such as glutaraldehyde), wherein the cells are characterised by marked swelling of the mitochondria and cytoplasm, followed by cell destruction and autolysis. Alternatively, the uncultured cell population is subjected may be contacted with antigen and subsequently subjected to apoptotic conditions. Cells expressing or presenting antigen can be induced to undergo apoptosis *in vitro* or *in vivo* using a variety of methods known in the art including, but not limited to, viral infection, irradiation with ultraviolet light, gamma radiation, steroids, fixing (e.g., with glutaraldehyde), cytokines or by depriving donor cells of nutrient's in the cell culture medium. Time course studies can establish incubation periods sufficient for optimal induction of apoptosis in a population of cells. For example, monocytes infected with influenza virus begin to express early markers for apoptosis by 6 hours after infection. Examples of specific markers for apoptosis include Annexin V, TUNEL+ cells, DNA laddering and uptake of propidium iodide.

[0099] According to this aspect of the present invention, the antigen used to contact the population is not limited to the overlapping set of peptides described in Section 2 above but instead

encompasses antigens of any biological type including, for example, simple intermediary metabolites, sugars, lipids, and hormones as well as macromolecules such as complex carbohydrates, phospholipids, nucleic acid molecules and proteinaceous molecules. In illustrative examples, the antigen corresponding to the target antigen is selected from whole protein antigens, cellular material (e.g., live or inactivated cancer cells), particulate matter such as, but not limited to, cell debris, apoptotic cells, lipid aggregates such as liposomes, membranous vehicles, microspheres, heat aggregated proteins, virosomes, virus-like particles and whole organisms including, for example, bacteria, mycobacteria, viruses, fungi, protozoa or parts thereof.

[0100] Target antigens may be selected from endogenous antigens produced by a host or exogenous antigens that are foreign to the host, as described for example in Section 2. In certain embodiments, the antigen corresponding to the target antigen is a proteinaceous antigen. Such antigens may be isolated from a natural source or may be prepared by recombinant techniques as known in the art. Alternatively, crude antigen preparations can be produced by isolating a sample of a cell population or tissue for which a modified immune response is desired, and either lysing the sample or subjecting the sample to conditions that will lead to the formation of apoptotic cells (e.g., irradiation with ultra violet or with gamma rays, viral infection, cytokines or by depriving cells of nutrients in the cell culture medium, incubation with hydrogen peroxide, or with drugs such as dexamethasone, ceramide chemotherapeutics and anti-hormonal agents such as Lupron™ or Tamoxifen™). The lysate or the apoptotic cells can then be used as a source of crude antigen for use in soluble form or for contact with antigen-presenting cells as described in more detail below.

### 3.1 Sources of antigen-presenting cells

[0101] The antigen-presenting cells suitably encompass both professional and facultative types of antigen-presenting cells. For example, professional antigen-presenting cells include, but are not limited to, macrophages, monocytes, cells of myeloid lineage, including monocytic-granulocytic-DC precursors, marginal zone Kupffer cells, microglia, T cells, B cells Langerhans cells and dendritic cells including interdigitating dendritic cells and follicular dendritic cells. Examples of facultative antigen-presenting cells include but are not limited to activated T cells, astrocytes, follicular cells, endothelium and fibroblasts. In a preferred embodiment, the antigen-presenting cells are selected from monocytes, macrophages, cells of myeloid lineage, dendritic cells or Langerhans cells.

[0102] Antigen-presenting cells or their precursors can be isolated by methods known to those of skill in the art. The source of antigen-presenting cell or precursor may differ depending upon the antigen-presenting cell required for modulating a specified immune response. In this context, the antigen-presenting cell can be selected from dendritic cells, macrophages, monocytes and other cells of myeloid lineage. Typically, precursors of antigen-presenting cells can be isolated from any tissue, but are most easily isolated from blood, cord blood or bone marrow (Sorg *et al.*, 2001, *Exp Hematol*

29: 1289-1294; Zheng *et al.*, 2000, *J Hematother Stem Cell Res* 9: 453-464). It is also possible to obtain suitable precursors from diseased tissues such as rheumatoid synovial tissue or fluid following biopsy or joint tap (Thomas *et al.*, 1994, *J Immunol* 152: 2613-2623; Thomas *et al.*, 1994, *J Immunol* 153: 4016-4028). Other examples include, but are not limited to liver, spleen, heart, kidney, gut and tonsil (Lu *et al.*, 1994, *Transplantation* 64: 1808-1815; McIlroy *et al.*, 2001, *Blood* 97: 3470-3477; Vremec *et al.*, 2000, *J Immunol* 164: 2978-2986; Hart and Fabre, 1981, *J Exp Med* 154(2): 347-361; Hart and McKenzie, 1988, *J Exp Med* 168(1): 157-170; Pavli *et al.*, 1990, *Immunology* 70(1): 40-47).

[0103] Leukocytes isolated directly from tissue provide a major source of antigen-presenting cell precursors. Typically, these precursors can only differentiate into antigen-presenting cells by culturing in the presence or absence of various growth factors *ex vivo* for at least about 6-9 days. However, in some advantageous embodiments of the present invention, antigen-presenting cells or their precursors (e.g., in the form of freshly isolated blood or PMBC) are simply isolated from an individual and incubated in the presence of antigen and preferably one or more growth factors for much shorter periods, e.g., less than about 48, 36, 24, 12, 8, 7, 6, 5, 4, 3 or 2 hours or even less than about 60, 50, 40, 30, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3 or 2 minutes, to produce antigen-specific antigen-presenting cells that are effective in raising an immunogenic response to that antigen.

[0104] In some embodiments, antigen-presenting cell precursors may be differentiated from crude mixtures or from partially or substantially purified preparations of precursors. Leukocytes can be conveniently purified from blood or bone marrow by density gradient centrifugation using, for example, Ficoll Hypaque which eliminates neutrophils and red cells (peripheral blood mononuclear cells or PBMCs), or by ammonium chloride lysis of red cells (leukocytes or white blood cells). Many precursors of antigen-presenting cells are present in peripheral blood as non-proliferating monocytes, which can be differentiated into specific antigen-presenting cells, including macrophages and dendritic cells, suitably by incubating the precursor in the presence of one or more specific cytokines.

[0105] Tissue-derived precursors such as unfractionated lymph node-derived mononuclear cells, precursors of tissue dendritic cells or of Langerhans cells are typically obtained by mincing tissue (e.g., basal layer of epidermis) and digesting it with collagenase or dispase followed by density gradient separation, or selection of precursors based on their expression of cell surface markers. For example, Langerhans cell precursors express CD1 molecules as well as HLA-DR and can be purified on this basis.

[0106] In some embodiments, the antigen-presenting cell precursor is a precursor of macrophages. Generally these precursors can be obtained from monocytes of any source and can be differentiated into macrophages by prolonged incubation in the presence of medium and macrophage colony stimulating factor (M-CSF) (Erickson-Miller *et al.*, 1990, *Int J Cell Cloning* 8: 346-356; Metcalf and Burgess, 1982, *J Cell Physiol* 111: 275-283).



[0107] In other embodiments, the antigen presenting cell precursor is a precursor of Langerhans cells. Usually, Langerhans cells can be generated from human monocytes or CD34<sup>+</sup> bone marrow precursors in the presence of granulocyte/macrophage colony-stimulating factor (GM-CSF), IL-4/TNF $\alpha$  and TGF $\beta$  (Geissmann *et al.*, 1998, *J Exp Med* 187: 961-966; Strobl *et al.*, 1997, *Blood* 90: 1425-1434 Strobl *et al.*, 1997, *Adv Exp Med Biol* 417: 161-165; Strobl *et al.*, 1996, *J Immunol* 157: 1499-1507).

[0108] In some embodiments, the antigen-presenting cell precursor is a precursor of dendritic cells. Several potential dendritic cell precursors can be obtained from peripheral blood, cord blood or bone marrow. These include monocytes, CD34<sup>+</sup> stem cells, granulocytes, CD33<sup>+</sup>CD11c<sup>+</sup> DC precursors, and committed myeloid progenitors – described below.

[0109] **Monocytes.** Monocytes can be purified by adherence to plastic for 1-2 h in the presence of tissue culture medium (e.g., RPMI) and serum (e.g., human or foetal calf serum), or in serum-free medium (Anton *et al.*, 1998, *Scand J Immunol* 47: 116-121.; Araki *et al.*, 2001, *Br J Haematol* 114: 681-689; Mackensen *et al.*, 2000, *Int J Cancer* 86: 385-392; Nestle *et al.*, 1998, *Nat Med* 4: 328-332; Romani *et al.*, 1996, *J Immunol Meth* 196: 137-151; Thurner *et al.*, 1999, *J Immunol Methods* 223: 1-15). Monocytes can also be elutriated from peripheral blood (Garderet *et al.*, 2001, *J Hematother Stem Cell Res* 10: 553-567). Monocytes can also be purified by immunoaffinity techniques, including immunomagnetic selection, flow cytometric sorting or panning (Araki *et al.*, 2001, *supra*; Battye and Shortman, 1991, *Curr. Opin. Immunol.* 3: 238-241), with anti-CD14 antibodies to obtain CD14<sup>hi</sup> cells. The numbers (and therefore yield) of circulating monocytes can be enhanced by the *in vivo* use of various cytokines including GM-CSF (Groopman *et al.*, 1987, *N Engl J Med* 317: 593-598; Hill *et al.*, 1995, *J Leukoc Biol* 58: 634-642). Monocytes can be differentiated into dendritic cells by prolonged incubation in the presence of GM-CSF and IL-4 (Romani *et al.*, 1994, *J Exp Med* 180: 83-93; Romani *et al.*, 1996, *supra*). A combination of GM-CSF and IL-4 at a concentration of each at between about 200 to about 2000 U/mL, more preferably between about 500 to about 1000 U/mL and even more preferably between about 800 U/mL (GM-CSF) and 1000 U/mL (IL-4) produces significant quantities of immature dendritic cells, i.e., antigen-capturing phagocytic dendritic cells. Other cytokines which promote differentiation of monocytes into antigen-capturing phagocytic dendritic cells include, for example, IL-13.

[0110] **CD34<sup>+</sup> stem cells.** Dendritic cells can also be generated from CD34<sup>+</sup> bone marrow derived precursors in the presence of GM-CSF, TNF $\alpha$   $\pm$  stem cell factor (SCF, c-kitL), or GM-CSF, IL-4  $\pm$  flt3L (Bai *et al.*, 2002, *Int J Oncol* 20: 247-53; Chen *et al.*, 2001, *Clin Immunol* 98: 280-292; Loudovaris *et al.*, 2001, *J Hematother Stem Cell Res* 10: 569-578). CD34<sup>+</sup> cells can be derived from a bone marrow aspirate or from blood and can be enriched as for monocytes using, for example, immunomagnetic selection or immunocolumns (Davis *et al.*, 1994, *J Immunol Meth* 175: 247-257). The proportion of CD34<sup>+</sup> cells in blood can be enhanced by the *in vivo* use of various cytokines



including (most commonly) G-CSF, but also flt3L and progenipoiectin (Fleming *et al.*, 2001, *Exp Hematol* 29: 943-951; Pulendran *et al.*, 2000, *J Immunol* 165: 566-572; Robinson *et al.*, 2000, *J Hematother Stem Cell Res* 9: 711-720).

[0111] **Other myeloid progenitors.** DC can be generated from committed early myeloid progenitors in a similar fashion to CD34<sup>+</sup> stem cells, in the presence of GM-CSF and IL-4/TNF. Such myeloid precursors infiltrate many tissues in inflammation, including rheumatoid arthritis synovial fluid (Santiago-Schwarz *et al.*, 2001, *J Immunol* 167(3): 1758-68). Expansion of total body myeloid cells including circulating dendritic cell precursors and monocytes, can be achieved with certain cytokines, including flt-3 ligand, granulocyte colony-stimulating factor (G-CSF) or progenipoiectin (pro-GP) (Fleming *et al.*, 2001, *supra*; Pulendran *et al.*, 2000, *supra*; Robinson *et al.*, 2000, *supra*). Administration of such cytokines for several days to a human or other mammal would enable much larger numbers of precursors to be derived from peripheral blood or bone marrow for *in vitro* manipulation. Dendritic cells can also be generated from peripheral blood neutrophil precursors in the presence of GM-CSF, IL-4 and TNF $\alpha$  (Kelly *et al.*, 2001, *Cell Mol Biol* (Noisy-le-grand) 47(1): 43-54; Oehler *et al.*, 1998, *J Exp Med.* 187(7):1019-28). It should be noted that dendritic cells can also be generated, using similar methods, from acute myeloid leukemia cells (Oehler *et al.*, 2000, *Ann Hematol* 79(7): 355-62).

[0112] **Tissue DC precursors and other sources of APC precursors.** Other methods for DC generation exist from, for example, thymic precursors in the presence of IL-3 +/- GM-CSF, and liver DC precursors in the presence of GM-CSF and a collagen matrix. Transformed or immortalised dendritic cell lines may be produced using oncogenes such as *v-myc* as for example described by (Paglia *et al.*, 1993, *J Exp Med* 178(6): 1893-901) or by *myb* (Banyer and Hapel, 1999, *J Leukoc Biol* 66(2): 217-223; Gonda *et al.*, 1993, *Blood* 82(9): 2813-2822).

[0113] **Circulating DC precursors.** These have been described in human and mouse peripheral blood. One can also take advantage of particular cell surface markers for identifying suitable dendritic cell precursors. Specifically, various populations of dendritic cell precursors can be identified in blood by the expression of CD11c and the absence or low expression of CD14, CD19, CD56 and CD3 (O'Doherty *et al.*, 1994, *Immunology* 82: 487-493; O'Doherty *et al.*, 1993, *J Exp Med* 178: 1067-1078). These cells can also be identified by the cell surface markers CD13 and CD33 (Thomas *et al.*, 1993, *J Immunol* 151(12): 6840-6852). A second subset, which lacks CD14, CD19, CD56 and CD3, known as plasmacytoid dendritic cell precursors, does not express CD11c, but does express CD123 (IL-3R chain) and HLA-DR (Farkas *et al.*, 2001, *Am J Pathol* 159: 237-243; Grouard *et al.*, 1997, *J Exp Med* 185: 1101-1111; Rissoan *et al.*, 1999, *Science* 283: 1183-1186). Most circulating CD11c<sup>+</sup> dendritic cell precursors are HLA-DR<sup>+</sup>, however some precursors may be HLA-DR<sup>-</sup>. The lack of MHC class II expression has been clearly demonstrated for peripheral blood dendritic cell precursors (del Hoyo *et al.*, 2002, *Nature* 415: 1043-1047).

[0114] Optionally, CD33<sup>+</sup>CD14<sup>-lo</sup> or CD11c<sup>+</sup>HLA-DR<sup>+</sup>, lineage marker-negative dendritic cell precursors described above can be differentiated into more mature antigen-presenting cells by incubation for 18-36 h in culture medium or in monocyte conditioned medium (Thomas *et al.*, 1993, *J Immunol* 151(12): 6840-6852; Thomas and Lipsky, 1994, *J Immunol* 153: 4016-4028; O'Doherty *et al.*, 1993, *supra*). Alternatively, following incubation of peripheral blood non-T cells or unpurified PBMC, the mature peripheral blood dendritic cells are characterised by low density and so can be purified on density gradients, including metrizamide and Nycodenz (Freudenthal and Steinman, 1990, *Proc Natl Acad Sci U S A* 87: 7698-7702; Vremec and Shortman, 1997, *J Immunol* 159: 565-573), or by specific monoclonal antibodies, such as but not limited to the CMRF-44 mAb (Fearnley *et al.*, 1999, *Blood* 93, 728-736; Vuckovic *et al.*, 1998, *Exp Hematol* 26: 1255-1264). Plasmacytoid dendritic cells can be purified directly from peripheral blood on the basis of cell surface markers, and then incubated in the presence of IL-3 (Grouard *et al.*, 1997, *supra*; Rissoan *et al.*, 1999, *supra*). Alternatively, plasmacytoid DC can be derived from density gradients or CMRF-44 selection of incubated peripheral blood cells as above.

[0115] In general, for dendritic cells generated from any precursor, when incubated in the presence of activation factors such as monocyte-derived cytokines, lipopolysaccharide and DNA containing CpG repeats, cytokines such as TNF- $\alpha$ , IL-6, IFN- $\alpha$ , IL-1 $\beta$ , necrotic cells, readherence, whole bacteria, membrane components, RNA or polyIC, immature dendritic cells will become activated (Clark, 2002, *J Leukoc Biol* 71: 388-400; Hacker *et al.*, 2002, *Immunology* 105: 245-251; Kaisho and Akira, 2002, *Biochim Biophys Acta* 1589: 1-13; Koski *et al.*, 2001, *Crit Rev Immunol* 21: 179-189).

[0116] Other methods for isolation, expansion and/or maturation of dendritic cells are described for example by Takamizawa *et al.* (1997, *J Immunol*, 158(5): 2134-2142), Thomas and Lipsky (1994, *J Immunol*, 153(9): 4016-4028), O'Doherty *et al.* (1994, *Immunology*, 82(3): 487-93), Fearnley *et al.* (1997, *Blood*, 89(10): 3708-3716), Weissman *et al.* (1995, *Proc Natl Acad Sci U S A*, 92(3): 826-830), Freudenthal and Steinman (1990, *Proc Natl Acad Sci U S A*, 87(19): 7698-7702), Romani *et al.* (1996, *J Immunol Methods*, 196(2): 137-151), Reddy *et al.* (1997, *Blood*, 90(9): 3640-3646), Thurnher *et al.* (1997, *Exp Hematol*, 25(3): 232-237), Caux *et al.* (1996, *J Exp Med*, 184(2): 695-706; 1996, *Blood*, 87(6): 2376-85), Luft *et al.* (1998, *Exp Hematol*, 26(6): 489-500; 1998, *J Immunol*, 161(4): 1947-1953), Cella *et al.* (1999, *J Exp Med*, 189(5): 821-829; 1997, *Nature*, 388(644): 782-787; 1996, *J Exp Med*, 184(2): 747-572), Ahonen *et al.* (1999, *Cell Immunol*, 197(1): 62-72) and Piemonti *et al.* (1999, *J Immunol*, 162(11): 6473-6481).

[0117] In certain embodiments, the antigen-presenting cells or their precursors are in the form of a substantially purified population of cells. In other embodiments, the antigen-presenting cells or their precursors are in the form of a heterogenous pool of cells. Suitably, the substantially purified or heterogenous population used to contact an antigen is in cultured or uncultured form as defined herein.

In certain advantageous embodiments employing an uncultured population of antigen-presenting cells or their precursors, the population can be incubated for short time periods (e.g., as low as about 5, 10, 15, 20, 20, 40, 50, 60 min) and the contacted population can be infused directly into a recipient without further culturing of the cells. This further shortens the processing time to permit potentially the harvesting of autologous or syngeneic antigen-presenting cells, treatment of those cells with antigen and infusion of the antigen-contacted cells into a patient in a single sitting or day.

### 3.2 Delivery of antigen to antigen-presenting cells

[0118] The delivery of exogenous antigen to antigen-presenting cells can be enhanced by methods known to practitioners in the art. For example, several different strategies have been developed for delivery of exogenous antigen to the endogenous processing pathway of antigen-presenting cells, especially dendritic cells. These methods include insertion of antigen into pH-sensitive liposomes (Zhou and Huang, 1994, *Immunomethods*, 4:229-235), osmotic lysis of pinosomes after pinocytic uptake of soluble antigen (Moore *et al.*, 1988, *Cell*, 54:777-785), coupling of antigens to potent adjuvants (Aichele *et al.*, 1990, *J. Exp. Med.*, 171: 1815-1820; Gao *et al.*, 1991, *J. Immunol.*, 147: 3268-3273; Schulz *et al.*, 1991, *Proc. Natl. Acad. Sci. USA*, 88: 991-993; Kuzu *et al.*, 1993, *Euro. J. Immunol.*, 23: 1397-1400; and Jondal *et al.*, 1996, *Immunity* 5: 295-302) and apoptotic cell delivery of antigen (Albert *et al.* 1998, *Nature* 392:86-89; Albert *et al.* 1998, *Nature Med.* 4:1321-1324; and in International Publications WO 99/42564 and WO 01/85207). Recombinant bacteria (eg. *E. coli*) or transfected host mammalian cells may be pulsed onto dendritic cells (as particulate antigen, or apoptotic bodies respectively) for antigen delivery. Recombinant chimeric virus-like particles (VLPs) have also been used as vehicles for delivery of exogenous heterologous antigen to the MHC class I processing pathway of a dendritic cell line (Bachmann *et al.*, 1996, *Eur. J. Immunol.*, 26(11): 2595-2600). In some embodiments, solubilized antigen (e.g., in DMSO) is incubated with antigen-presenting cells.

[0119] Alternatively, or in addition, an antigen (e.g., a peptide antigen) may be linked to, or otherwise associated with, a cytolysin to enhance the transfer of the peptide into the cytosol of an antigen-presenting cell of the invention for delivery to the MHC class I pathway. Exemplary cytolysins include saponin compounds such as saponin-containing Immune Stimulating Complexes (ISCOMs) (see e.g., Cox and Coulter, 1997, *Vaccine* 15(3): 248-256 and U.S. Patent No. 6,352,697), phospholipases (see, e.g., Camilli *et al.*, 1991, *J. Exp. Med.* 173: 751-754), pore-forming toxins (e.g., an alpha-toxin), natural cytolysins of gram-positive bacteria, such as listeriolysin O (LLO, e.g., Mengaud *et al.*, 1988, *Infect. Immun.* 56: 766-772 and Portnoy *et al.*, 1992, *Infect. Immun.* 60: 2710-2717), streptolysin O (SLO, e.g., Palmer *et al.*, 1998, *Biochemistry* 37(8): 2378-2383) and perfringolysin O (PFO, e.g., Rossjohn *et al.*, *Cell* 89(5): 685-692). Where the antigen-presenting cell is phagosomal, acid activated cytolysins may be advantageously used. For example, listeriolysin exhibits greater pore-forming ability at mildly acidic pH (the pH conditions within the phagosome), thereby



facilitating delivery of vacuole (including phagosome and endosome) contents to the cytoplasm (see, e.g., Portnoy *et al.*, *Infect. Immun.* 1992, 60: 2710-2717).

[0120] The amount of antigen to be placed in contact with antigen-presenting cells can be determined empirically by persons of skill in the art. The antigen-presenting cells should be exposed to the antigen for a period of time sufficient for those cells to present the peptides or processed forms thereof for the modulation of T cells. In some advantageous embodiments the antigen-presenting cells are incubated in the presence of antigen for less than about 48, 36, 24, 12, 8, 7, 6, 5, 4, 3 or 2 hours or even for less than about 60, 50, 40, 30, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3 or 2 minutes). The time and dose of peptides necessary for the cells to optionally process and present the peptides or their processed forms may be determined using pulse-chase protocols in which exposure to peptides is followed by a washout period and exposure to a read-out system e.g., antigen reactive T cells. Once the optimal time and dose necessary for cells to express the peptides or their processed forms on their surface is determined, a protocol may be used to prepare cells and peptides for inducing immunogenic responses. Those of skill in the art will recognise in this regard that the length of time necessary for an antigen-presenting cell to present an antigen on its surface may vary depending on the antigen or form of antigen employed, its dose, and the antigen-presenting cell employed, as well as the conditions under which antigen loading is undertaken. These parameters can be determined by the skilled artisan using routine procedures. Efficiency of priming of the antigen-presenting cells can be determined by assaying T cell cytotoxic activity *in vitro* or using antigen-presenting cells as targets of CTLs. Other methods known to practitioners in the art, which can detect the presence of antigen on the surface of antigen-presenting cells after exposure to one or more of the modified and unmodified antigens, are also contemplated by the presented invention.

[0121] Usually, about 0.1 to 20 µg/mL of antigen (e.g., peptide antigen) to about 1-10 million antigen-presenting cells is suitable for producing primed antigen-specific antigen-presenting cells. Typically antigen-presenting cells are incubated with antigen for about 1 to 6 hr at 37° C, although it is also possible to expose antigen-presenting cells to antigen for the duration of incubation with one or more growth factors. As discussed above, the present inventors have shown that successful presentation of antigen (e.g., peptide antigen) or their processed forms can be achieved using much shorter periods of incubation (e.g., about 5, 10, 15, 20, 30, 40, 50 minutes) using antigen at a concentration of about 10-20 µg/mL.

[0122] If desired, all or a portion of the antigen-presenting cells can be frozen in an appropriate cryopreservative solution, until required. For example, the cells may be diluted in an appropriate medium, such as one containing 10% of autologous serum + 10% of dimethylsulfoxide in a phosphate buffer saline. In certain embodiments, the cells are conserved in a dehydrated form.



#### 4. *Lymphocyte embodiments*

[0123] The antigen-presenting cells of the invention may be obtained or prepared to contain and/or express one or more antigens by any number of means, such that the antigen(s) or processed form(s) thereof, is (are) presented by those cells for potential modulation of other immune cells, including T lymphocytes and B lymphocytes, and particularly for producing T lymphocytes and B lymphocytes that are primed to respond to a specified antigen or group of antigens. In some embodiments, the subject antigen-presenting cells are useful for producing primed T lymphocytes to an antigen or group of antigens. The efficiency of inducing lymphocytes, especially T lymphocytes, to exhibit an immune response to a specified antigen can be determined by any suitable method including, but not limited to, assaying T lymphocyte cytolytic activity *in vitro* using for example antigen-specific antigen-presenting cells as targets of antigen-specific cytolytic T lymphocytes (CTL); assaying antigen-specific T lymphocyte proliferation (see, e.g., Vollenweider and Groseurth, 1992, *J. Immunol. Meth.* 149: 133-135), measuring B cell response to the antigen using, for example, ELISPOT assays, and ELISA assays; interrogating cytokine profiles; or measuring delayed-type hypersensitivity (DTH) responses by test of skin reactivity to a specified antigen (see, e.g., Chang *et al.* (1993, *Cancer Res.* 53: 1043-1050). Other methods known to practitioners in the art, which can detect the presence of antigen on the surface of antigen-presenting cells after exposure to the antigen, are also contemplated by the present invention.

[0124] Accordingly, the present invention also provides antigen-specific B or T lymphocytes, especially T lymphocytes, which respond in an antigen-specific fashion to representation of the antigen. In some embodiments, antigen-specific T lymphocytes are produced by contacting an antigen-presenting cell as defined above with a population of T lymphocytes, which may be obtained from any suitable source such as spleen or tonsil/lymph nodes but is preferably obtained from peripheral blood. The T lymphocytes can be used as crude preparations or as partially purified or substantially purified preparations, which are suitably obtained using standard techniques as, for example, described in "Immunochemical Techniques, Part G: Separation and Characterization of Lymphoid Cells" (*Meth. in Enzymol.* 108, Edited by Di Sabato *et al.*, 1984, Academic Press). This includes rosetting with sheep red blood cells, passage across columns of nylon wool or plastic adherence to deplete adherent cells, immunomagnetic or flow cytometric selection using appropriate monoclonal antibodies is known in the art.

[0125] The preparation of T lymphocytes is contacted with the antigen-presenting cells of the invention for an adequate period of time for priming the T lymphocytes to the antigen or antigens presented by those antigen-presenting cells. This period will preferably be at least about 1 day, and up to about 5 days.

[0126] In some embodiments, a population of antigen-presenting cells is cultured in the presence of a heterogeneous population of T lymphocytes, which is suitably obtained from peripheral blood,

together with a set of peptides of the invention corresponding to an antigen to which an immune response is required. These cells are cultured for a period of time and under conditions sufficient for the peptides, or their processed forms, to be presented by the antigen-presenting cells; and the antigen-presenting cells to prime a subpopulation of the T lymphocytes to respond to the antigen.

5 **5. Cell based therapy or prophylaxis**

[0127] The antigen-presenting cells described in Section 3 and the lymphocytes described in Section 4 can be administered to a patient, either by themselves or in combination, for modulating an immune response, especially for modulating an immune response to one or more cognate antigens. These cell based compositions are useful, therefore, for treating or preventing a disease or condition as noted above. The cells of the invention can be introduced into a patient by any means (e.g., injection), which produces the desired immune response to an antigen or group of antigens. The cells may be derived from the patient (i.e., autologous cells) or from an individual or individuals who are MHC matched or mismatched (i.e., allogeneic) with the patient. Typically, autologous cells are injected back into the patient from whom the source cells were obtained. The injection site may be subcutaneous, intraperitoneal, intramuscular, intradermal, intravenous or intralymphoid. The cells may be administered to a patient already suffering from a disease or condition or who is predisposed to a disease or condition in sufficient number to treat or prevent or alleviate the symptoms of the disease or condition. The number of cells injected into the patient in need of the treatment or prophylaxis may vary depending on *inter alia*, the antigen or antigens and size of the individual. This number may range for example between about  $10^3$  and  $10^{11}$ , and usually between about  $10^5$  and  $10^7$  cells (e.g., in the form blood, PMBC or purified dendritic cells or T lymphocytes). Single or multiple (2, 3, 4 or 5) administrations of the cells can be carried out with cell numbers and pattern being selected by the treating physician. The cells should be administered in a pharmaceutically acceptable carrier, which is non-toxic to the cells and the individual. Such carrier may be the growth medium in which the cells were grown, or any suitable buffering medium such as phosphate buffered saline. The cells may be administered alone or as an adjunct therapy in conjunction with other therapeutics known in the art for the treatment or prevention of unwanted immune responses for example but not limited to glucocorticoids, methotrexate, D-penicillamine, hydroxychloroquine, gold salts, sulfasalazine, TNF-alpha or interleukin-1 inhibitors, and/or other forms of specific immunotherapy.

30 **6. Compositions**

[0128] The overlapping sets of peptides described in Sections 2 and the antigen-primed antigen-presenting cells described in Section 3 or the lymphocytes described in Section 4 (therapeutic/prophylactic agents) can be used singly or together as active ingredients for the treatment or prophylaxis of various conditions associated with the presence of one or more target polypeptide antigens. These therapeutic/prophylactic agents can be administered to a patient either by themselves,

or in compositions where they are mixed with a suitable pharmaceutically acceptable carrier and/or diluent, or an adjuvant.

[0129] The invention also encompasses a method for stimulating a patient's immune system, and preferably for eliciting a humoral and/or cellular immune response to a polypeptide of interest, by administering to the patient a therapeutic agent or composition as described above. Such stimulation may be utilised for the treatment and/or prophylaxis of a disease or condition including, but not restricted to, a pathogenic infection (e.g., viral, bacterial, fungal, protozoan) or a cancer. Accordingly, the invention contemplates a method for treatment and/or prophylaxis of a disease or condition, comprising administering to a patient in need of such treatment a therapeutically/prophylactically effective amount of a therapeutic agent or composition as broadly described above.

[0130] Depending on the specific conditions being treated, therapeutic/prophylactic agents may be formulated and administered systemically or locally. Techniques for formulation and administration may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, Pa., latest edition. Suitable routes may, for example, include oral, rectal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections. For injection, which constitutes one desirable embodiment of the present invention, the therapeutic agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art. Intra-muscular and subcutaneous injection is appropriate, for example, for administration of immunogenic compositions, vaccines and DNA vaccines. In certain embodiments of the present invention, the immunogenic compositions are administered intravenously.

[0131] The therapeutic/prophylactic agents can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. Such carriers enable the compounds of the invention to be formulated in dosage forms such as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. These carriers may be selected from sugars, starches, cellulose and its derivatives, malt, gelatine, talc, calcium sulphate, vegetable oils, synthetic oils, polyols, alginic acid, phosphate buffered solutions, emulsifiers, isotonic saline, and pyrogen-free water.

[0132] Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. The dose of agent administered to a patient should be sufficient to effect a beneficial response in the patient over time such as a reduction in the symptoms associated with the condition.



The quantity of the therapeutic/prophylactic agent(s) to be administered may depend on the subject to be treated inclusive of the age, sex, weight and general health condition thereof. In this regard, precise amounts of the therapeutic/prophylactic agent(s) for administration will depend on the judgement of the practitioner. In determining the effective amount of the agent to be administered in the treatment or prophylaxis of the condition, the physician may evaluate tissue levels of a target antigen, and progression of the disease or condition. In any event, those of skill in the art may readily determine suitable dosages of the therapeutic agents of the invention.

[0133] Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilisers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

[0134] Pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Such compositions may be prepared by any of the methods of pharmacy but all methods include the step of bringing into association one or more therapeutic agents as described above with the carrier which constitutes one or more necessary ingredients. In general, the pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilising processes.

[0135] Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterise different combinations of active compound doses.



[0136] Pharmaceutical which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticiser, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilisers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilisers may be added.

[0137] Dosage forms of the therapeutic agents of the invention may also include injecting or implanting controlled releasing devices designed specifically for this purpose or other forms of implants modified to act additionally in this fashion. Controlled release of an agent of the invention may be effected by coating the same, for example, with hydrophobic polymers including acrylic resins, waxes, higher aliphatic alcohols, polylactic and polyglycolic acids and certain cellulose derivatives such as hydroxypropylmethyl cellulose. In addition, controlled release may be effected by using other polymer matrices, liposomes and/or microspheres.

[0138] Therapeutic agents of the invention may be provided as salts with pharmaceutically compatible counterions. Pharmaceutically compatible salts may be formed with many acids, including but not limited to hydrochloric, sulphuric, acetic, lactic, tartaric, malic, succinic, *etc.* Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms.

[0139] For any compound used in the method of the invention, the effective dose can be estimated initially from cell culture assays. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes the IC<sub>50</sub> as determined in cell culture (e.g., the concentration of a test agent, which achieves a half-maximal reduction in target antigen). Such information can be used to more accurately determine useful doses in humans.

[0140] Toxicity and therapeutic efficacy of the compounds of the invention can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD<sub>50</sub> (the dose lethal to 50% of the population) and the ED<sub>50</sub> (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD<sub>50</sub>/ED<sub>50</sub>. Compounds that exhibit large therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED<sub>50</sub> with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilised. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See for example Fingl *et al.*, 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p1).

[0141] Dosage amount and interval may be adjusted individually to provide plasma levels of the active compound(s) which are sufficient to maintain target antigen-reducing effects or effects that ameliorate the disease or condition. Usual patient dosages for systemic administration range from 1-2000 mg/day, commonly from 1-250 mg/day, and typically from 10-150 mg/day. Stated in terms of patient body weight, usual dosages range from 0.02-25 mg/kg/day, commonly from 0.02-3 mg/kg/day, typically from 0.2-1.5 mg/kg/day. Stated in terms of patient body surface areas, usual dosages range from 0.5-1200 mg/m<sup>2</sup>/day, commonly from 0.5-150 mg/m<sup>2</sup>/day, typically from 5-100 mg/m<sup>2</sup>/day.

[0142] Alternately, one may administer the agent in a local rather than systemic manner, for example, *via* injection of the compound directly into a tissue, often in a depot or sustained release formulation. Furthermore, one may administer the agent in a targeted drug delivery system, for example, in a liposome coated with tissue-specific antibody. The liposomes will be targeted to and taken up selectively by the tissue.

[0143] From the foregoing, it will be appreciated that the agents of the invention may be used as therapeutic or prophylactic immunomodulating compositions or vaccines. Accordingly, the invention extends to the production of immunomodulating compositions containing as active compounds one or more of the therapeutic/prophylactic agents of the invention. Any suitable procedure is contemplated for producing such vaccines. Exemplary procedures include, for example, those described in NEW GENERATION VACCINES (1997, Levine *et al.*, Marcel Dekker, Inc. New York, Basel Hong Kong).

[0144] Immunomodulating compositions according to the present invention can contain a physiologically acceptable diluent or excipient such as water, phosphate buffered saline and saline. They may also include an adjuvant as is well known in the art. Suitable adjuvants include, but are not limited to: surface active substances such as hexadecylamine, octadecylamine, octadecyl amino acid esters, lysolecithin, dimethyldioctadecylammonium bromide, N, N-dioctadecyl-N', N'-bis(2-hydroxyethyl-propanediamine), methoxyhexadecylglycerol, and pluronic polyols; polyamines such as pyran, dextran sulfate, poly IC carbopol; peptides such as muramyl dipeptide and derivatives, dimethylglycine, tuftsin; oil emulsions; and mineral gels such as aluminum phosphate, aluminum hydroxide or alum; lymphokines, QuilA and immune stimulating complexes (ISCOMS).

[0145] The antigen-primed antigen-presenting cells of the invention and antigen-specific T lymphocytes generated with these antigen-presenting cells, as described *supra*, can be used as active compounds in immunomodulating compositions for prophylactic or therapeutic applications. In some embodiments, the antigen-primed antigen-presenting cells of the invention are useful for generating large numbers of CD8<sup>+</sup> or CD4<sup>+</sup> CTL, for adoptive transfer to immunosuppressed individuals who are unable to mount normal immune responses. For example, antigen-specific CD8<sup>+</sup> CTL can be adoptively transferred for therapeutic purposes in individuals afflicted with HIV infection (Koup *et al.*, 1991, *J. Exp. Med.*, 174: 1593-1600; Carmichael *et al.*, 1993, *J. Exp. Med.*, 177: 249-256; and Johnson

*et al.*, 1992, *J. Exp. Med.*, 175: 961-971), malaria (Hill *et al.*, 1992, *Nature*, 360: 434-439) and malignant tumours such as melanoma (Van der Brogen *et al.*, 1991, *Science*, 254: 1643-1647; and Young and Steinman, 1990, *J. Exp. Med.*, 171: 1315-1332).

[0146] In other embodiments, the immunomodulating composition of the invention is suitable for treatment or prophylaxis of a cancer. Cancers which could be suitably treated in accordance with the practices of this invention include cancers associated with a viral infection such as cervical cancer (e.g., papillomavirus infection) and Burkitt's lymphoma (e.g., Epstein Barr virus infection). Other virus associated cancers include, but are not restricted to, HTLV1 associated leukemia, Non Hodgkins lymphoma (EBV), anal cancer, skin cancer (HPV), hepatocellular carcinoma (HBV) and Kaposi sarcoma (HHV8). Alternatively, the cancer may be a non-virally associated cancer such as but not limited to melanoma, lung cancer, breast cancer, prostate cancer, colon cancer, pancreatic cancer, stomach cancer, bladder cancer, kidney cancer, post transplant lymphoproliferative disease (PTLD), Hodgkin's Lymphoma and the like.

[0147] In still other embodiments, the immunomodulating composition is suitable for treatment or prophylaxis of a viral, bacterial or protozoan infection. Viral infections contemplated by the present invention include, but are not restricted to, infections caused by HIV, Hepatitis, Influenza, Japanese encephalitis virus, Epstein-Barr virus and respiratory syncytial virus. Bacterial infections include, but are not restricted to, those caused by *Neisseria* species, *Meningococcal* species, *Haemophilus* species, *Salmonella* species, *Streptococcal* species, *Legionella* species and *Mycobacterium* species. Protozoan infections encompassed by the invention include, but are not restricted to, those caused by *Plasmodium* species (e.g., malaria), *Schistosoma* species (e.g., schistosomiasis), *Leishmania* species, *Trypanosoma* species, *Toxoplasma* species and *Giardia* species.

## 7. Methods for assessing immunomodulation

[0148] The effectiveness of the immunisation may be assessed using any suitable technique. An individual's capacity to respond to foreign or disease-specific antigens (e.g., viral antigens and cancer antigens) may be determined by assessing whether those cells primed to attack such antigens are increased in number, activity, and ability to detect and destroy those antigens. Strength of immune response is measured by standard tests including: direct measurement of peripheral blood lymphocytes by means known to the art; natural killer cell cytotoxicity assays (see, e.g., Provinciali M. *et al* (1992, *J. Immunol. Meth.* 155: 19-24), cell proliferation assays (see, e.g., Vollenweider, I. and Groseurth, P. J. (1992, *J. Immunol. Meth.* 149: 133-135), immunoassays of immune cells and subsets (see, e.g., Loeffler, D. A., *et al.* (1992, *Cytom.* 13: 169-174); Rivoltini, L., *et al.* (1992, *Can. Immunol. Immunother.* 34: 241-251); or skin tests for cell-mediated immunity (see, e.g., Chang, A. E. *et al* (1993, *Cancer Res.* 53: 1043-1050). Alternatively, the efficacy of the immunisation may be monitored using one or more techniques including, but not limited to, HLA class I tetramer staining - of both



fresh and stimulated PBMCs (see for example Allen *et al.*, *supra*), proliferation assays (Allen *et al.*, *supra*), ELISPOT assays and intracellular cytokine staining (Allen *et al.*, *supra*), ELISA Assays - for linear B cell responses; and Western blots of cell sample expressing the synthetic polynucleotides. Particularly relevant will be the cytokine profile of T cells activated by antigen, and more particularly the production and secretion of IFN  $\gamma$ , IL-2, IL4, IL5, IL-10, TGF $\beta$  and TNF  $\alpha$ .

[0149] The cytotoxic activity of T lymphocytes, and in particular the ability of cytotoxic T lymphocytes to be induced by antigen-presenting cells, may be assessed by any suitable technique known to those of skill in the art. For example, a sample comprising T lymphocytes to be assayed for cytotoxic activity is obtained and the T lymphocytes are then exposed to antigen-primed antigen-presenting cells, which have been caused to present antigen. After an appropriate period of time, which may be determined by assessing the cytotoxic activity of a control population of T lymphocytes which are known to be capable of being induced to become cytotoxic cells, the T lymphocytes to be assessed are tested for cytotoxic activity in a standard cytotoxic assay.

[0150] The method of assessing CTL activity is particularly useful for evaluating an individual's capacity to generate a cytotoxic response against cells expressing tumour or viral antigens. Accordingly, this method is useful for evaluating an individual's ability to mount an immune response to a cancer or virus. For example, CTL lysis assays may be employed using stimulated splenocytes or peripheral blood mononuclear cells (PBMC) on peptide coated or recombinant virus infected cells using  $^{51}\text{Cr}$  labelled target cells. Such assays can be performed using for example primate, mouse or human cells (Allen *et al.*, 2000, *J. Immunol.* 164(9): 4968-4978 also Woodberry *et al.*, *infra*). In addition, CTL activity can be measured in outbred primates using the *in vivo* detection method described in Figure 1.. In this method, autologous cells (e.g., PMBC) are labelled with an optically detectable label (e.g., a fluorescent, chemiluminescent or phosphorescent or visual label or dye) and are contacted with one ore more peptide sets as disclosed herein. The peptide sets are chosen so that they correspond to an antigen which is the subject of a CTL response under test in a subject. The autologous cells are infused into the subject and lymphocytes from the subject are harvested after a suitable period to permit the subject's immune system sufficient time to respond to the autologous cells (e.g., 10 minutes to 24 hours post infusion). The harvested lymphocytes are then analysed to identify the number or proportion of lymphocytes which contain or otherwise carry the optically detectable label, which represents a measure of the *in vivo* CTL response to the antigen in the subject.

[0151] In order that the invention may be readily understood and put into practical effect, particular preferred embodiments will now be described by way of the following non-limiting examples.



## EXAMPLES

### EXAMPLE 1

#### **In vivo cytotoxic T-lymphocyte killing**

[0152] The standard measure of virus-specific CTL effector is measured *via* the release of a radioisotope  $^{51}\text{Cr}$  from target cells, an assay that is tedious and poorly sensitive. By pulsing dye-labelled autologous macaque PBMC with large pools of SIV and SHIV overlapping peptides (OPAL) and infusing the cells back into the same animal, the inventors were able to kinetically show SHIV-specific killing in blood sampled at various time-points following the infusion of OPAL by flow cytometry.

10 [0153] Two weeks after full immunisation (week 10), three of four immunised animals displayed moderate to large (11.4-76%) killing of gag-pulsed PBMC by 16 hours post-OPAL infusion, whereas control-immunised monkeys displayed <7% gag-specific killing. One immunised animal, monkey H20, demonstrated vigorous gag-specific killing (27.3%) as early as 4 hours post-infusion (Figure 2). These data were consistent with T cell responses induced by the vaccines as analysed by IFN $\gamma$  ELISpot and ICS (data not shown), indicating the usefulness of OPAL to measure effective CTL effector responses primed by the DNA and FPV vaccines.

[0154] Shortly (2 weeks) after SHIV intrarectal challenge all four immunised animals exhibited large degrees of gag-specific killing (65-98.3%) 16 hours post-OPAL infusion, and two of four (monkeys H20 and H21) further demonstrated >99% pol-specific killing (Figure 3). In comparison with control-immunised animals, monkey E20 displayed <6% killing of both gag- and pol-pulsed PBMC whereas monkey E22 showed >90% and 31.9% of gag- and pol-pulsed PBMC, respectively. Interestingly, the animals that displayed moderate to high degrees of pol-specific killing (monkeys H20, H21 and E22) were also the only animals that had previously received 2 doses of infused pol-pulsed PBMC (weeks 10 and 15), whereas monkeys B00, H8 and E20 received pol-pulsed PBMC only once prior. This observation suggests that the infusion of OPAL may have: (a) boosted pol-specific T cell responses primed by the vaccines that were weakly or not detected by IFN $\gamma$  ELISpot and ICS (data not shown), and; (b) induced pol-specific immunity in naïve animals evident post-SHIV challenge.

### EXAMPLE 2

30 **Analysis of the immunogenicity induced by infusing peptide-pulsed autologous cells.**

[0155] It seemed plausible that if *in vivo* CTL killing could be efficiently measured by OPAL infusion, this method may be able to either prime a new or boost an existing immune response. IFN $\gamma$  ELISpot and ICS assays were therefore performed prior to- and one week following each OPAL

infusion assay to analyse whether there would be an increase in T cell immunogenicity previously primed by the vaccines or by the OPAL infusion method itself (Figure 4).

5 [0156] Following the first OPAL infusion performed at week 10, a 3- to 16-fold increase in IFN $\gamma$ -secreting cells to SIV gag peptide pool was detected in monkeys H20 and H21, measuring up to 430 spot-forming cells (Figure 5). Monkey H8 measured a 54% increase to 215 spot-forming cells, whereas no increase was measured in control-immunised animals. Analysis of monkeys B00 (post-OPAL infusion) and E20 (pre-OPAL infusion) for all antigens analysed were excluded due to developmental problems of the assay. Of the four animals that received pol-pulsing at week 10, monkeys H20, H21 and E22, displayed increased pol responses by up to 140 spot-forming cells post-10 OPAL infusion, whereas no significant ELISpot responses were detected in monkey E20. No nef-specific T cell was in all animals apparent before or after OPAL-infusion. These results suggest a boosting effect in T cell immunogenicity following gag- and pol-peptide pulsing in the animals previously primed for SIVgag/pol responses, and furthermore indicate priming for SIVpol in a naïve animal (monkey E22).

15 [0157] At week 15, 8 weeks following full immunisation, a second OPAL infusion assay was performed in the six animals. ELISpot analyses revealed increased responses to gag peptide pool by up to 500 spot-forming cells from approximately 50 or less spot-forming cells prior to OPAL infusion in the four animals pre-immunised with DNA and FPV vaccines. In control-immunised animals, no gag-specific T cells were measured before or after the assay (Figure 6). In comparison, a slight increase in 20 pol-specific responses (up to 40 spot-forming cells) from baseline was measured in only a few animals. Large increased responses to WI SIV were measured in all pre-immunised animals (up to 450 spot-forming cells), whereas control-immunised animals displayed modest or no increases (up to 50 spot-forming cells). All responses to SIV nef and SHIV env were minimal or undetected in all animals prior to and after OPAL infusion.

25 [0158] Following SHIV intrarectal challenge, all animals except monkey E20 displayed increased gag responses measuring between 50-600 spot-forming cells. Similar responses were observed for WI SIV but to levels up to 200 spot-forming cells, whereas pol responses above 50 spot-forming cells were only evident in monkey H20.

30 [0159] The immunogenicity of OPAL infusion was further verified by comparison to animals that received the same immunisation regimen but did not receive OPAL infusion (Figure 7). No rise in SIV gag, pol or WI SIV-specific T cells were detected in groups 1 (control-immunised) and 2 (2 $\times$  DNA/FPV-immunised) from weeks 9 to 11 and 15 to 18. Responses from weeks 20 to 21 increased slightly the groups, attributable to responses enhanced by SHIV challenge at week 18.

35 [0160] The experiments performed on macaques infused with peptide pulsed whole blood also demonstrated a boost in CD4+ and CD8+ T cell responses to both (a) several parts of SHIV in

recipients of SHIV-peptide pulsed blood (Figure 9), (b) 2 pools of HCV peptides spanning the entire HCV genome in recipients of HCV-peptide pulsed blood (Figure 10), and (c) a pool of peptides spanning known HIV-1 drug resistant mutations in recipients of autologous blood pulsed with HIV-1 resistant peptides (Figure 11).

5

### EXAMPLE 3

#### **Outcome of SHIV<sub>mn229</sub> intrarectal challenge**

[0161] The highly pathogenic SHIV<sub>mn229</sub> challenge stock was inoculated intrarectally into all macaques 10 weeks after full immunisation at a dose of  $10^5$  TCID<sub>50</sub>. Plasma SHIV RNA and CD4+ T cell counts were followed in all control-and 2×DNA/FPV-immunised animals (Figure 8).

10 [0162] Control-immunised monkeys E20 and E22 exhibited peak viral loads of  $7.8 \pm 0.7 \log_{10}$  copies/mL at 2 weeks following challenge. The peak viral load of monkey E20 may have occurred between week 1 and 2, however, set-point levels of both monkeys (measured 5 to 11 weeks post challenge) remained high at  $5.9 \pm 0.3 \log_{10}$  copies/mL. Conversely at week 2, CD4+ T cell counts dropped dramatically to  $1.6 \pm 1.1\%$  of total lymphocytes, and set-point levels were steady at  $0.3 \pm 0.2\%$ .  
15 Monkeys that received the same immunisations but no OPAL infusions (group 1) performed only marginally worse than monkeys E20 and E22 in terms of peak and set-point viral loads ( $8.2 \pm 0.1 \log_{10}$  copies/mL and  $6.2 \pm 0.3 \log_{10}$  copies/mL), as well as CD4+ counts (set-point  $0.5 \pm 0.3\%$ ).

Based on the enhanced pol-specific killing that may have been attributed to 2 separate OPAL infusions, the SHIV viral loads and CD4+ T cell counts of monkeys H20 and H21 were compared to  
20 monkeys B00 and H8 that received only 1 dose of pol-OPAL infusions. Peak viral load of monkeys H20 and H21 (receiving 2 pol-OPAL infusions) was at least 10-fold lower than monkeys B00 and H8 ( $5.9 \pm 1.3$  vs.  $7.1 \pm 0.4 \log_{10}$  copies/mL,  $P=0.08$ ), and set-point viral load showed a trend towards being lower ( $4.1 \pm 0.9$  vs.  $5.4 \pm 0.7 \log_{10}$  copies/mL,  $P=0.08$ , student's *t* test). Incidentally, set-point CD4+ T cell count for monkeys H20 and H21 was significantly greater than monkeys B00 and H8 ( $18.9 \pm 6.1\%$   
25 vs.  $8.4\%$ ,  $P=0.02$ ). Although statistically insignificant in comparison with group 2 animals who received the same immunisations but no OPAL infusions ( $P=0.12$ ), monkeys H20 and H21 that received multiple pol-OPAL infusions displayed a trend towards the retainment of CD4+ T cells although viral loads were relatively similar, indicative of viral challenge protection. Set-point CD4+ T cell count and viral load of group 2 were  $13.0 \pm 3.7\%$  and  $4.8 \pm 0.2 \log_{10}$  copies/mL, respectively.

30 [0163] In comparison to control-immunised monkeys E20 and E22, both set-point viral load and CD4+ T cell count of monkeys H20 and H21 were significantly different ( $P=0.01$ ,  $P=0.00$ ). The set-point viral load of monkeys B00 and H8, on the other hand, was not significantly lower than monkeys E20 and E22 ( $P=0.37$ ) despite significant set-point levels of CD4+ T cells ( $P=0.01$ ). Note that monkey

H20 had completely cleared plasma viral RNA from week 5 and onwards and retained CD4+ T cells at normal levels.

#### DISCUSSION OF THE EXAMPLES

[0164] The vital role for HIV-1-specific CD4+ T-helper (Th) and CD8+ CTL responses in controlling HIV-1 replication is the focus of many current vaccine concepts. The infusion of autologous PBMC pulsed with large overlapping sets of SHIV 15mer peptides (OPAL) was surprisingly immunogenic in its ability to boost SHIV-specific immune responses as analysed by IFN $\gamma$  ELISpot and ICS assays. This finding forms the potential basis of a novel vaccine or immunotherapeutic strategy as described herein.

[0165] The evidence for this immunogenicity of peptide-pulsed fresh PBMC was five-fold: (a) Increases in SIV gag-specific IFN $\gamma$  ELISpot responses were observed one week after each of the 3 SIV gag OPAL infusions (week 10, 15, and 20) in all vaccinated monkeys. In contrast, at week 10 and 15, SIVgag responses in equivalently immunised animals (group 2) not receiving the OPAL infusion significantly declined. (b) Increases in SIV pol-specific IFN $\gamma$  ELISpot responses were observed in immunised animals one week following the SIV pol infusion at week 10 and 20. Interestingly this was observed in only the two monkeys H20 and H21 that received multiple SIV pol OPAL infusions prior to SHIV challenge (weeks 10 and 15) and not in animals receiving SIV pol peptide pulsed cells at week 15. This is of particular interest since the pol-specific T cell responses to the DNA and FPV vaccines alone were modest or undetectable by ELISpot and ICS. (c) High levels of SIV pol-specific *in vivo* killing were also seen in the two monkeys that received 2 prior infusions of SIV pol OPAL infusions. (d) This immunogenicity data was further confirmed by high levels of SIV pol-specific IFN $\gamma$  intracellular cytokine responses in the two immunised animals receiving the multiple SIV pol OPAL infusions. (e) There was a trend towards greater protection from SHIV challenge in animals receiving multiple OPAL infusions. Together, these results suggest that pulsing autologous PBMC *ex vivo* with pools of overlapping peptides is an effective method for boosting immune responses. In addition, data show that peptide pulsed whole blood can both stimulate T cell responses to several parts of SHIV in recipients of SHIV-peptide pulsed blood, as well as induce *de novo* T cell responses to (a) 2 pools of HCV peptides spanning the entire HCV genome in recipients of HCV-peptide pulsed blood and (b) a pool of peptides spanning known HIV-1 drug resistant mutations in recipients of autologous blood pulsed with HIV-1 resistant peptides.

[0166] There is a body of data ascertaining the use of pulsing autologous or syngeneic cells with defined peptide epitopes or whole antigen for the induction (or 'cross-priming') of immune responses (22, 23, 27, 34, 35). The use of specialised antigen presenting cells such as monocyte-derived dendritic cells pulsed with, for example, single tumour antigens or whole inactivated SIV has also been studied extensively as an immunotherapeutic tool (36-39). However, to the inventors' knowledge this is the



first report of utilising large peptide pools spanning an entire protein (125 SIV gag 15mers or 263 SIV pol 15mers) and the use of whole PBMC cultured for short periods *ex vivo*, as a method of boosting immune responses.

[0167] In one control-immunised animal, monkey E22, which received multiple infusions of PMBC pulsed with SIV pol (and SIV gag), a modest induction of SIV gag and SIV pol-specific IFN $\gamma$  ELISpot responses was detected. This animal subsequently had high levels of SIV gag- and pol-specific killing analysed at week 20, presumably from the boosting effect of the SHIV challenge. The efficiency of priming an immune response by OPAL infusion therefore seems feasible. These data were confirmed when whole blood was pulsed with HCV or HIV-1 drug resistant peptides, which efficiently induced high levels of CD4+ and CD8+ T cell responses as assessed by ICS. These data also demonstrate the feasibility of using whole blood as an antigen-presenting cell (APC) source, which would be more practical than PBMC or other more complex APC preparations (such as monocyte-derived dendritic cells) in the field.

[0168] Further modifications to the OPAL technique, such as the enrichment for APC and/or dendritic cells (DC) (40), would potentially enhance the immunogenicity of OPAL infusion as a therapeutic vaccine since DC cultured from PBMC of HIV-infected patients (41, 42) and SIV-infected animals (40) can elicit potent T-cell responses. Alternatively, the prospect of using whole blood rather than PBMC fractions as a means of delivering OPAL will certainly benefit a clinical setting, particularly for HIV-infected persons. Furthermore, a smaller whole blood sample may not require as high a concentration of peptide since 1  $\mu$ g/mL is effective *in vitro* for whole blood analysis by ICS. It is also conceivable that direct intravenous infection of pooled peptides would mimic the immunogenicity of the OPAL effect. The use of consensus HIV-1 clade peptide sets of gag and pol offers the broad epitopic breadth desired of an effective therapeutic vaccine for humans. The immunogenicity of antigens that regulate viral replication, such as rev, tat, vpu, vif and vpr, which are poorly immunogenic by current vaccine approaches, should also be improved using this strategy. In addition, the general method of using blood or PBMC or other uncultured APC-containing fraction directly as an APC source immediately suggests the possibility of pulsing other sources of antigen (including but not limited to whole protein, DNA, live vector vaccines or cancer cell preparations) onto such APC populations prior to infusion. It is believed that such antigen-loaded cell APC populations could be more immunogenic (presumably by binding directly to abundant APCs) than administering the antigen by other common methods such as intramuscularly (where few APCs exist).

## EXAMPLE 4

### MATERIALS AND METHODS

#### Animals

[0169] Male juvenile, colony-bred pigtailed macaques (*Macaca nemestrina*, aged 2-4 years) were studied. All animals were housed under PC3 biosafety conditions by trained animal technicians at the CSIRO Australian Animal Health Laboratory, Geelong. Prior to all procedures, animals were anaesthetised with ketamine (10 mg/kg, intramuscularly). Health and weight were routinely monitored. All conditions and protocols were approved by the CSIRO animal health and the University of Melbourne animal ethics committees.

#### Pre-immunisations

[0170] To evaluate whether the OPAL method could boost T cell responses in animals with pre-primed responses. T cell responses were induced in macaques by administering 2 DNA vaccines expressing HIV or SIV structural genes followed by a FPV boost vaccine expressing similar HIV or SIV genes as previously described (16). DNA vaccines in saline were administered twice intramuscularly (0.5 mL to each anterior quadracep) at a dose of 1mg/dose. FPV boosts were delivered intramuscularly a dose of  $5 \times 10^7$  pfu.

#### Isolation of plasma and peripheral blood mononuclear cells (PBMC) from whole blood

[0171] Blood was collected in 9 mL Na<sup>+</sup> Heparin and 3 mL EDTA vacutainers from the femoral vein of each animal on study weeks prior to and after vaccination and SHIV challenge. Plasma samples were removed following centrifugation (800×g, room temperature, RT, 8 min; Beckman Coulter) and stored in 3×1.5-mL tubes at -70° C. Plasma collected in EDTA-anticoagulated blood was used for RNA extraction. Media (RPMI-1640 supplemented with penicillin, streptomycin and glutamine; Invitrogen) equal to the volume of plasma collected was added to the blood and mixed prior to PBMC isolation on Ficoll-Paque, used according to the manufacturer's instructions (Amersham Pharmacia). PBMC were washed twice (500×g, 10° C, 6min) and resuspended in 1 mL media for counting (Beckman Coulter Counter®) in preparation of immunological assays.

#### Overlapping peptides

[0172] 15-mer peptides (>80% purity) overlapping by 11 amino acids spanning the entire gag (125 peptides), pol (260 peptides) and nef (21 peptides) of SIV<sub>mac239</sub> and env (211 peptides) protein of SHIV<sub>SF162P3</sub> (NIH AIDS Research and Reference Reagent depository) (Tables 1-4) were pooled for each protein by solubilising each 1mg peptide aliquot in 10-40 µL of DMSO to final concentrations: SIV<sub>mac239</sub> gag (670 µg/mL or 730 µg/mL); pol (304 µg/mL), and; nef (4.762 mg/mL), and; SHIV<sub>SF162P3</sub> env (330 µg/mL), stored at -70° C until use. 18mer peptides overlapping by 11 amino acids spanning

the entire HCV open reading frames (NIH AIDS Research and Reference Reagent depository) were pooled into 2 pools (HCV1 and HCV2) encompassing the structural and regulatory genes of HCV. Non-overlapping 17mer peptides spanning known sites of HIV-1 drug resistance mutations were specifically designed and purchased from Mimotopes Australia (Figure 12).

5

*SIV antigens for in vitro analyses*

[0173] Whole inactivated SIV (WI SIV) and its control (supernatant from Hut78-CLE cell-line used to culture the WI SIV) (AIDS Vaccine Program, National Cancer Institute, MD) were stored at -70° C until use.

*In vivo cytotoxic T lymphocyte killing*

10 [0174] At weeks 10, 15 and 20 following the initial vaccination, PBMC from the macaques were isolated from 40-50 mL blood, as described above. 25 mL sterile injectable saline was infused into the animals immediately after blood sampling to prevent hypovolemia. PBMC were resuspended in PBS and divided into 3 or 4 equal volumes, 0.5 mL. Cells were pulsed with SIVgag, pol, nef or SHIVenv peptide pools (10 µg/mL) or DMSO (volume of equal to the volume of SIVgag), in PBS for 90 min at  
15 37° C, or on ice, with regular mixing. To subsequently track each peptide-pulsed cell population by flow cytometry, each peptide/DMSO-pulsed population was then labelled with a concentration of CFSE or SNARF (Molecular Probes). 5 mM CFSE stock in DMSO at-20° C was thawed and diluted in PBS. Neat SNARF stock was dissolved in 83 µLDMSO to make 1mM and diluted in PBS. Table 1 shows the final concentrations of each dye. Cells were mixed thoroughly and stained for 10 min in a  
20 37° C waterbath, followed by one wash in RF5 then PBS (500×g, 10°C, 6min). All peptide/DMSO-pulsed cells for each animal were pooled in 1.5 mL saline for re-infusion into the femoral vein. 3 mL blood was sampled from the opposite femoral vein at 5 min, and at 4 and 16hr following infusion. Red blood cells were lysed with 10 mL FACS Lysing Solution (BD Biosciences), incubated for 10min at room temp. Cells were pelleted and washed twice with PBS (800×g, RT, 7min), and fixed with 1-2  
25 mL 2% paraformaldehyde (Figure 1).

[0175] To determine whether cell populations were being selectively killed, 10<sup>6</sup> events gated live lymphocytes were collected by flow cytometry (FACSort Calibre, BD). CFSE and SNARF fluorescence were detected by FL1 and FL2 channels, respectively. For analysis, killing was expressed as the percentage of target versus control peptide-pulsed cell clearance. In the event of acquiring  
30 unequal labelled populations by flow cytometry at 5 minutes post-OPAL infusion, the degree of killing was subsequently scaled with respect to the initial population ratios obtained at 5 minutes. PBMC were also analysed prior to, and following, OPAL-infusion by IFNγ ELISpot and ICS to detect whether T cell immune responses were enhanced.

SHIV challenge of macaques

[0176] To assess the efficacy to the vaccines, each macaque was inoculated intrarectally with SHIV<sub>nm229</sub> ( $5 \times 10^4$  TCID<sub>50</sub>/mL on CD8-depleted *M. nemestrina* PBMC) in 0.5 mL doses over 2 days (total  $10^5$  TCID<sub>50</sub>/mL) 18 weeks after the initial immunisation, as previously described (32).

5 Quantification of viral SHIV RNA by reverse-transcriptase real-time PCR

[0177] RNA extraction: To detect SHIV RNA in macaques following SHIV challenge, total RNA was initially extracted from stored plasma samples from anti-coagulated blood collected in EDTA with QIAamp® Viral RNA commercial kit (Qiagen) as previously described (32). Briefly, plasma samples were centrifuged (500×g, RT, 10min) to remove cells (preventing DNA contamination). 140 µL plasma RNA coupled to Carrier RNA in Buffer AVL and 96-100% ethanol was centrifuged and bound to a filter membrane. 60 µL RNA was eluted with Buffer AW1 and AW2 through a spin column. All reagents except ethanol supplied by kit.

[0178] Reverse-transcriptase PCR: 10 µL RNA was then reverse transcribed into cDNA, in duplicate, with the reaction mixture (20 µL): 2.9 µL RNase/DNase-free water (Promega); 3 µL 10× TaqMan buffer A (Applied Biosystems); 6 µL MgCl<sub>2</sub> (25nM) (Applied Biosystems); 1.5 µL Random Hexamers (diluted 1/2; Applied Biosystems); 6µl dNTPs (2.5nM; Promega); 1.5 µL; Promega); 0.5 µL Rnasin (40 U/mL; Promega); 0.1 µL MMLV-RT superscript (200U/mL; Invitrogen), for one thermal cycle: 25° C (15min) → 42° C (40min) → 75° C (5min) (GeneAmp PCR System 9700, Applied Biosystems). A third test per sample was set up to assess the presence of SHIV DNA contamination, using the same reaction mix excluding MMLV-RT superscript. SIV RNA standards (33) were serially diluted and reverse-transcribed in duplicate (limit of detection, 1500 copies/mL).

[0179] Real-time PCR: cDNA was amplified with reaction mixture (20 µl): 141µl RNase/DNase-free water (Promega); 2µL 10× PCR buffer II (Applied Biosystems); 1 µL MgCl<sub>2</sub> (Applied Biosystems); 1 µL SL03 SIVgag (20pmol/µL); 1µL SL04 SIVgag (20 pmol/□L); 0.3 µL SL07 molecular beacon 0.5µL Tag Gold (Applied Biosystems) as previously described (33). Reaction temperature was initially raised and held at 95 °C for 10 min to activate Tag Gold enzyme, followed by 45 thermal cycles: 95° C (15 sec) → 55° C (30 sec) → 72 °C (30 sec). Real-time analysis was performed on amplicon detection at 55° C (30 sec) stages by Sequence Detector software v1.6.3 (Applied Biosystems).

30 CD4+ T cell counts

[0180] To assess the depletion of CD4+ T cells following SHIV challenge, 200 µL whole blood was incubated with 5 µL PE-conjugated anti-human CD3, 5 µL FITC-conjugated anti-human CD4, 5



5  $\mu$ L PerCP-conjugated anti-human CD8 (clone SP34; L200, and; Leu-2a, respectively; BD Pharmingen) monoclonal antibodies for 20 min in dark, RT. Red blood cells were lysed with 2 mL FACS Lysing Solution (BD Biosciences) and fixed as described in method 2.8. 50,000 total events were collected by 3-colour FACScan Calibre® and CD4+ and CD8+ T cell counts expressed as the percentage of gated lymphocytes.

*Analysis of stimulation or induction of SHIV, HCV and peptides derived from resistant HIV-1 strains by the whole blood OPAL technique*

10 [0181] In a separate experiment to assess (a) whether peptide-pulsed whole blood (as compared to PBMC which had be used previously) could be effectively used as an immune stimulant and (b) whether the OPAL technique could stimulate *de novo*, un-primed, immune responses, selected SHIV-infected macaques were infused with either whole blood pulsed at 5  $\mu$ g/mL for 1hr with either a series of overlapping 15mer SHIV peptides (3 pools) or a series of overlapping 18mer HCV peptides (2 pools) and a series of non-overlapping 17mer peptides encompassing known mutations induced by HIV-1 drugs as illustrated in Figures 9-12.

15 [0182] The disclosure of every patent, patent application, and publication cited herein is hereby incorporated herein by reference in its entirety.

[0183] The citation of any reference herein should not be construed as an admission that such reference is available as "Prior Art" to the instant application.

20 [0184] Throughout the specification the aim has been to describe the preferred embodiments of the invention without limiting the invention to any one embodiment or specific collection of features. Those of skill in the art will therefore appreciate that, in light of the instant disclosure, various modifications and changes can be made in the particular embodiments exemplified without departing from the scope of the present invention. All such modifications and changes are intended to be included within the scope of the appended claims.

## TABLES

TABLE 1

One embodiment of an SIV<sub>mac236</sub> gag peptide pool sequence. Each peptide is 15 amino acids in length and overlaps the preceding peptide by 11 amino acids. Peptide 125 is 14 amino acids in length. The full-length gag sequence [SEQ ID NO:2184] is modified from the HIV sequence database <http://hiv-web.lanl.gov>.

#	PEPTIDE	SEQUENCE ID
1	MGVRNSVLSGKKADE	SEQ ID NO:1
2	NSVLSGKKADELEKI	SEQ ID NO:2
3	SGKKADELEKIRLRP	SEQ ID NO:3
4	ADELEKIRLRPNGKK	SEQ ID NO:4
5	EKIRLRPNGKKKYML	SEQ ID NO:5
6	LRPNGKKKYMLKHVV	SEQ ID NO:6
7	GKKKYMLKHVVWAAN	SEQ ID NO:7
8	YMLKHVVWAANELDR	SEQ ID NO:8
9	HVVWAANELDRFGLA	SEQ ID NO:9
10	AANELDRFGLAESLL	SEQ ID NO:10
11	LDRFGLAESLLENKE	SEQ ID NO:11
12	GLAESLLENKEGCQK	SEQ ID NO:12
13	SLLENKEGCQKILSV	SEQ ID NO:13
14	NKEGCQKILSVLAPL	SEQ ID NO:14
15	CQKILSVLAPLVPTG	SEQ ID NO:15
16	LSVLAPLVPTGSEN	SEQ ID NO:16
17	LSVLAPLVPTGSEN	SEQ ID NO:17
18	PTGSENKSLYNTVC	SEQ ID NO:18
19	ENLKSLYNTVCVIWC	SEQ ID NO:19
20	SLYNTVCVIWCIHAE	SEQ ID NO:20
21	TVCVIWCIHAEKVK	SEQ ID NO:21
22	IWCIHAEKVKHTEE	SEQ ID NO:22
23	HAEEKVKHTEEAQOI	SEQ ID NO:23
24	KVKHTEEAQIVQRH	SEQ ID NO:24
25	TEEAQIVQRHLVVE	SEQ ID NO:25
26	KQIVQRHLVVETGTT	SEQ ID NO:26
27	QRHLVVETGTTETMP	SEQ ID NO:27
28	VVETGTTETMPKTSR	SEQ ID NO:28
29	GTTETMPKTSRPTAP	SEQ ID NO:29
30	TMPKTSRPTAPSSGR	SEQ ID NO:30

#	PEPTIDE	SEQUENCE ID
31	TSRPTAPSSGRGGNY	SEQ ID NO:31
32	TAPSSGRGGNYPVQQ	SEQ ID NO:32
33	SGRGGNYPVQQIGGN	SEQ ID NO:33
34	GNYPVQQIGGNYVHL	SEQ ID NO:34
35	VQQIGGNYVHLPLSP	SEQ ID NO:35
36	GGNYVHLPLSPRTL	SEQ ID NO:36
37	VHLPLSPRTLNAWVK	SEQ ID NO:37
38	LSPRTLNAWVKLIEE	SEQ ID NO:38
39	TLNAWVKLIEEKKFG	SEQ ID NO:39
40	WVKLIEEKKFGAEV	SEQ ID NO:40
41	IEEKKFGAEVPGFQ	SEQ ID NO:41
42	KFGAEVPGFQALSE	SEQ ID NO:42
43	EVVPGFQALSEGCTP	SEQ ID NO:43
44	GFQALSEGCTPYDIN	SEQ ID NO:44
45	LSEGCTPYDINQMLN	SEQ ID NO:45
46	CTPYDINQMLNCVGD	SEQ ID NO:46
47	DINQMLNCVGDHQA	SEQ ID NO:47
48	MLNCVGDHQAAMQII	SEQ ID NO:48
49	VGDHQAAMQIIRDII	SEQ ID NO:49
50	QAAMQIIRDIINEEA	SEQ ID NO:50
51	QIIRDIINEEAADWD	SEQ ID NO:51
52	DIINEEAADWDLQHP	SEQ ID NO:52
53	EEAADWDLQHPQAP	SEQ ID NO:53
54	DWDLQHPQAPQOGQ	SEQ ID NO:54
55	QHPQAPQOGQLREP	SEQ ID NO:55
56	PAPQOGQLREPSGSD	SEQ ID NO:56
57	QGQLREPSGSDIAGT	SEQ ID NO:57
58	REPSGSDIAGTTSSV	SEQ ID NO:58
59	GSDIAGTTSSVDEQI	SEQ ID NO:59
60	AGTTSSVDEQIQWY	SEQ ID NO:60

#	PEPTIDE	SEQUENCE ID
61	SSVDEQIQWMYRQQN	SEQ ID NO:61
62	EQIQWMYRQQNPIPV	SEQ ID NO:62
63	WMYRQQNPIPVGNIY	SEQ ID NO:63
64	QQNPIPVGNIYRRWI	SEQ ID NO:64
65	IPVGNIYRRWIQLGL	SEQ ID NO:65
66	NIYRRWIQLGLQKCV	SEQ ID NO:66
67	RWIQLGLQKCVRMYN	SEQ ID NO:67
68	LGLQKCVRMYNPTNI	SEQ ID NO:68
69	KCVRMYNPTNILDVK	SEQ ID NO:69
70	MYNPTNILDVKQGP	SEQ ID NO:70
71	TNILDVKQGPKEPFQ	SEQ ID NO:71
72	DVKQGPKEPFQSYVD	SEQ ID NO:72
73	GPKEPFQSYVDRFYK	SEQ ID NO:73
74	PFQSYVDRFYKSLRA	SEQ ID NO:74
75	YVDRFYKSLRAEQTD	SEQ ID NO:75
76	FYKSLRAEQTDAAVK	SEQ ID NO:76
77	LRAEQTDAAVKNWMT	SEQ ID NO:77
78	QTDAAVKNWMTQTLL	SEQ ID NO:78
79	AVKNWMTQTLLIQNA	SEQ ID NO:79
80	WMTQTLLIQNANPDC	SEQ ID NO:80
81	TLLIQNANPDCKLVL	SEQ ID NO:81
82	QNANPDCKLVLKGLG	SEQ ID NO:82
83	PDCKLVLKGLGVNPT	SEQ ID NO:83
84	LVLKGLGVNPTLEEM	SEQ ID NO:84
85	GLGVNPTLEEMLTAC	SEQ ID NO:85
86	NPTLEEMLTACQGVG	SEQ ID NO:86
87	EEMLTACQGVGGPGQ	SEQ ID NO:87
88	TACQGVGGPGQKARL	SEQ ID NO:88
89	GVGGPGQKARLMAEA	SEQ ID NO:89
90	PGQKARLMAEALKEA	SEQ ID NO:90
91	ARLMAEALKEALAPV	SEQ ID NO:91
92	AEALKEALAPVPIPF	SEQ ID NO:92
93	KEALAPVPIPFAAAQ	SEQ ID NO:93
94	APVPIPFAAAQQRGP	SEQ ID NO:94
95	IPFAAAQQRGPRKPI	SEQ ID NO:95
96	AAQQRGPRKPIKCWN	SEQ ID NO:96
97	RGPRKPIKCWNCGKE	SEQ ID NO:97
98	KPIKCWNCGKEGHS	SEQ ID NO:98
99	CWNCGKEGHSARQCR	SEQ ID NO:99
100	GKEGHSARQCRAPRR	SEQ ID NO:100

#	PEPTIDE	SEQUENCE ID
101	HSARQCRAPRRQGCW	SEQ ID NO:101
102	QCRAPRRQGCWKCGK	SEQ ID NO:102
103	PRRQGCWKCGKMDHV	SEQ ID NO:103
104	GCWKCGKMDHVMAC	SEQ ID NO:104
105	CGKMDHVMACPDRO	SEQ ID NO:105
106	DHVMACPDROAGFL	SEQ ID NO:106
107	AKCPDROAGFLGLGP	SEQ ID NO:107
108	DRQAGFLGLGPWGKK	SEQ ID NO:108
109	GFLGLGPWGKKPRNF	SEQ ID NO:109
110	LGPWGKKPRNFPMAQ	SEQ ID NO:110
111	GKKPRNFPMAQVHQG	SEQ ID NO:111
112	RNFPMAQVHQGLMPT	SEQ ID NO:112
113	MAQVHQGLMPTAPPE	SEQ ID NO:113
114	HQGLMPTAPPEDPAV	SEQ ID NO:114
115	MPTAPPEDPAVDLLK	SEQ ID NO:115
116	PPEDPAVDLLKNYMQ	SEQ ID NO:116
117	PAVDLLKNYMQLGKQ	SEQ ID NO:117
118	LLKNYMQLGKQQREK	SEQ ID NO:118
119	YMQLGKQQREKQRES	SEQ ID NO:119
120	GKQQREKQRESREKP	SEQ ID NO:120
121	REKQRESREKPYKEV	SEQ ID NO:121
122	RESREKPYKEVTEDL	SEQ ID NO:122
123	EKPYKEVTEDLLHLN	SEQ ID NO:123
124	KEVTEDLLHLNSLFG	SEQ ID NO:124
125	EDLLHLNSLFGGDQ	SEQ ID NO:125

TABLE 2

One embodiment of an SIV<sub>mac236</sub> pol peptide pool sequence. Each peptide is 15 amino acids in length and overlaps the preceding peptide by 11 amino acids. The full-length pol sequence [SEQ ID NO:2185] is modified from the HIV sequence database <http://hiv-web.lanl.gov>.

#	PEPTIDE	SEQUENCE ID
1	VLELWERGTLCKAMQ	SEQ ID NO:126
2	WERGTLCKAMQSPKK	SEQ ID NO:127
3	TLCKAMQSPKKTGML	SEQ ID NO:128
4	AMQSPKKTGMLEMWK	SEQ ID NO:129
5	PKKTGMLEMWKNGPC	SEQ ID NO:130
6	GMLEMWKNGPCYQOM	SEQ ID NO:131
7	MWKNGPCYQOMPRQT	SEQ ID NO:132
8	GPCYQOMPRQTGGFF	SEQ ID NO:133
9	GQMPRQTGGFFRPWS	SEQ ID NO:134
10	RQTGGFFRPWSMGKE	SEQ ID NO:135
11	GFFRPWSMGKEAPQF	SEQ ID NO:136
12	PWSMGKEAPQFPHGS	SEQ ID NO:137
13	GKEAPQFPHGSSASG	SEQ ID NO:138
14	PQFPHGSSASGADAN	SEQ ID NO:139
15	HGSSASGADANCSPR	SEQ ID NO:140
16	ASGADANCSPRGPSC	SEQ ID NO:141
17	DANCSPRGPSCGSAK	SEQ ID NO:142
18	SPRGPSCGSAKELHA	SEQ ID NO:143
19	PSCGSAKELHAVGQA	SEQ ID NO:144
20	SAKELHAVGQAAERK	SEQ ID NO:145
21	LHAVGQAAERKAERK	SEQ ID NO:146
22	GQAAERKAERKQREA	SEQ ID NO:147
23	ERKAERKQREALQGG	SEQ ID NO:148
24	ERKQREALQGDRGF	SEQ ID NO:149
25	REALQGDRGFAPQ	SEQ ID NO:150
26	QGDRGFAPQFSLW	SEQ ID NO:151
27	RGFAAPQFSLWRRPV	SEQ ID NO:152
28	APQFSLWRRPVVTAH	SEQ ID NO:153
29	SLWRRPVVTAHIEGQ	SEQ ID NO:154
30	RPVVTAHIEGQPVEV	SEQ ID NO:155
31	TAHIEGQPVEVLLDT	SEQ ID NO:156
32	EGQPVEVLLDTGADD	SEQ ID NO:157
33	VEVLLDTGADDSIVT	SEQ ID NO:158

#	PEPTIDE	SEQUENCE ID
34	LDTGADDSIVTGIEL	SEQ ID NO:159
35	ADDSIVTGIELGPHY	SEQ ID NO:160
36	IVTGIELGPHYTPKI	SEQ ID NO:161
37	IELGPHYTPKIVGGI	SEQ ID NO:162
38	PHYTPKIVGGIGGFI	SEQ ID NO:163
39	PKIVGGIGGFINTKE	SEQ ID NO:164
40	GGIGGFINTKEYKNV	SEQ ID NO:165
41	GFINTKEYKNVEIEV	SEQ ID NO:166
42	TKEYKNVEIEVLGKR	SEQ ID NO:167
43	KNVEIEVLGKRIKGT	SEQ ID NO:168
44	IEVLGKRIKGTIMTG	SEQ ID NO:169
45	GKRIKGTIMTGDTPI	SEQ ID NO:170
46	KGTIMTGDTPINIFG	SEQ ID NO:171
47	MTGDTPINIFGRNLL	SEQ ID NO:172
48	TPINIFGRNLLTALG	SEQ ID NO:173
49	IFGRNLLTALGMSLN	SEQ ID NO:174
50	NLLTALGMSLNFPIA	SEQ ID NO:175
51	ALGMSLNFPIAKVEP	SEQ ID NO:176
52	SLNFPIAKVEPVKVA	SEQ ID NO:177
53	PIAKVEPVKVALKPG	SEQ ID NO:178
54	VEPVKVALKPGKDG	SEQ ID NO:179
55	KVALKPGKDGPKLKQ	SEQ ID NO:180
56	KPGKDGPKLKQWPLS	SEQ ID NO:181
57	DGPKLKQWPLSKEKI	SEQ ID NO:182
58	LKQWPLSKEKIVALR	SEQ ID NO:183
59	PLSKEKIVALREICE	SEQ ID NO:184
60	EKIVALREICEKMEK	SEQ ID NO:185
61	ALREICEKMEKDGQL	SEQ ID NO:186
62	ICEKMEKDGQLEEAP	SEQ ID NO:187
63	MEKDGQLEEAPPTNP	SEQ ID NO:188
64	GQLEEAPPTNPYNT	SEQ ID NO:189
65	EAPPTNPYNTPTFAI	SEQ ID NO:190
66	TNPYNTPTFAIKKID	SEQ ID NO:191



#	PEPTIDE	SEQUENCE ID
67	NTPTFAIKKKDKNKW	SEQ ID NO:192
68	FAIKKKDKNKWRMLI	SEQ ID NO:193
69	KKDKNKWRMLIDFRE	SEQ ID NO:194
70	NKWRMLIDFRELN RV	SEQ ID NO:195
71	MLIDFRELN RV TQDF	SEQ ID NO:196
72	FRELN RV TQDFTEVQ	SEQ ID NO:197
73	NRVTQDFTEVQLGIP	SEQ ID NO:198
74	QDFTEVQLGIPHPAG	SEQ ID NO:199
75	EVQLGIPHPAGLAKR	SEQ ID NO:200
76	GIPHPAGLAKRK RIT	SEQ ID NO:201
77	PAGLAKRK RITVLDI	SEQ ID NO:202
78	AKRK RITVLDIGDAY	SEQ ID NO:203
79	RITVLDIGDAYFSIP	SEQ ID NO:204
80	LDIGDAYFSIPLDEE	SEQ ID NO:205
81	DAYFSIPLDEEFRQY	SEQ ID NO:206
82	SIPLDEEFRQYTAFT	SEQ ID NO:207
83	DEEFRQYTAFTLPSV	SEQ ID NO:208
84	RQYTAFTLPSVNNAE	SEQ ID NO:209
85	AFTLPSVNNAEPGKR	SEQ ID NO:210
86	PSVNNAEPGKRYIYK	SEQ ID NO:211
87	NAEPGKRYIYKVLPQ	SEQ ID NO:212
88	GKRYIYKVLPQG WKG	SEQ ID NO:213
89	IYKVLPQG WKGSPAI	SEQ ID NO:214
90	LPQG WKGSPAI FQYT	SEQ ID NO:215
91	WKGSPAI FQYTMRHV	SEQ ID NO:216
92	PAI FQYTMRHVLEPF	SEQ ID NO:217
93	QYTMRHVLEPF RKAN	SEQ ID NO:218
94	RHVLEPF RKANPDVT	SEQ ID NO:219
95	EPFRKANPDVTLVQY	SEQ ID NO:220
96	KANPDVTLVQYMDDI	SEQ ID NO:221
97	DVTLVQYMDDILIAS	SEQ ID NO:222
98	VQYMDDILIASDRTD	SEQ ID NO:223
99	DDILIASDRTDLEHD	SEQ ID NO:224
100	IASDRTDLEHDRVVL	SEQ ID NO:225
101	RTDLEHDRVVLQSKE	SEQ ID NO:226
102	EHDRVVLQSKELLNS	SEQ ID NO:227
103	VVLQSKELLNSIGFS	SEQ ID NO:228
104	SKELLNSIGFSTPEE	SEQ ID NO:229
105	LNSIGFSTPEEK FQK	SEQ ID NO:230
106	GFSTPEEK FQKDPFF	SEQ ID NO:231

#	PEPTIDE	SEQUENCE ID
107	PEEK FQKDPFFQWMG	SEQ ID NO:232
108	FQKDPFFQWMGYELW	SEQ ID NO:233
109	PPFQWMGYELWPTKW	SEQ ID NO:234
110	WMGYELWPTKWKLQK	SEQ ID NO:235
111	ELWPTKWKLQKIELP	SEQ ID NO:236
112	TKWKLQKIELPQRET	SEQ ID NO:237
113	LQKIELPQRETWTVN	SEQ ID NO:238
114	ELPQRETWTVNDIQK	SEQ ID NO:239
115	RETWTVNDIQKLVGV	SEQ ID NO:240
116	TVNDIQKLVGVLNWA	SEQ ID NO:241
117	IQKLVGVLNWAAQIY	SEQ ID NO:242
118	VGVLNWAAQIYPGIK	SEQ ID NO:243
119	NWAAQIYPGIKTKHL	SEQ ID NO:244
120	QIYPGIKTKHL CRLI	SEQ ID NO:245
121	GIKTKHL CRLIRGKM	SEQ ID NO:246
122	KHL CRLIRGKMTLTE	SEQ ID NO:247
123	RLIRGKMTLTEEVQW	SEQ ID NO:248
124	GKMTLTEEVQWTEMA	SEQ ID NO:249
125	LTEEVQWTEMAEAEY	SEQ ID NO:250
126	VQWTEMAEAEYEENK	SEQ ID NO:251
127	EMAEAEYEENKIILS	SEQ ID NO:252
128	AEYEENKIILSQEQE	SEQ ID NO:253
129	ENKIILSQEQEGCYY	SEQ ID NO:254
130	ILSQEQEGCYYQEGK	SEQ ID NO:255
131	EQEGCYYQEGKPLEA	SEQ ID NO:256
132	CYYQEGKPLEATVIK	SEQ ID NO:257
133	EGKPLEATVIKSQDN	SEQ ID NO:258
134	LEATVIKSQDNQWSY	SEQ ID NO:259
135	VIKSQDNQWSYKIHQ	SEQ ID NO:260
136	QDNQWSYKIHQEDKI	SEQ ID NO:261
137	WSYKIHQEDKILKVG	SEQ ID NO:262
138	IHQEDKILKVGKFAK	SEQ ID NO:263
139	DKILKVGKFAKIKNT	SEQ ID NO:264
140	KVGKFAKIKNTH TNG	SEQ ID NO:265
141	FAKIKNTH TNGVRLI	SEQ ID NO:266
142	KNTH TNGVRLLAHVI	SEQ ID NO:267
143	TNGVRLLAHVIQKIG	SEQ ID NO:268
144	RLLAHVIQKIGKEAI	SEQ ID NO:269
145	HVIQKIGKEAIVIWG	SEQ ID NO:270
146	KIGKEAIVIWGQVPK	SEQ ID NO:271

#	PEPTIDE	SEQUENCE ID
147	EAIVIWGQVPKFHLP	SEQ ID NO:272
148	IWGQVPKFHLPVEKD	SEQ ID NO:273
149	VPKFHLPVEKDVWEQ	SEQ ID NO:274
150	HLPVEKDVWEQWWT	SEQ ID NO:275
151	EKDVWEQWWTDYWQV	SEQ ID NO:276
152	WEQWWTDYWQVTWIP	SEQ ID NO:277
153	WTDYWQVTWIPEWDF	SEQ ID NO:278
154	WQVTWIPEWDFISTP	SEQ ID NO:279
155	WIPEWDFISTPPLVR	SEQ ID NO:280
156	WDFISTPPLVRLVFN	SEQ ID NO:281
157	STPPLVRLVFNLVKD	SEQ ID NO:282
158	LVRLVFNLVKDPLEG	SEQ ID NO:283
159	VFNLVKDPLEGGEETY	SEQ ID NO:284
160	VKDPLEGGEETYTDG	SEQ ID NO:285
161	IEGEETYTDGSCNK	SEQ ID NO:286
162	ETYYTDGSCNKQSKE	SEQ ID NO:287
163	TDGSCNKQSKEGKAG	SEQ ID NO:288
164	CNKQSKEGKAGYITD	SEQ ID NO:289
165	SKEGKAGYITDRGKD	SEQ ID NO:290
166	KAGYITDRGKDKVKV	SEQ ID NO:291
167	ITDRGKDKVKVLEQT	SEQ ID NO:292
168	GKDKVKVLEQTTNQQ	SEQ ID NO:293
169	VKVLEQTTNQQAELE	SEQ ID NO:294
170	EQTTNQQAELEAFLM	SEQ ID NO:295
171	NQQAELEAFLMALTD	SEQ ID NO:296
172	ELEAFLMALTDGPK	SEQ ID NO:297
173	FLMALTDGPKANII	SEQ ID NO:298
174	LTDGPKANIIVDSQ	SEQ ID NO:299
175	GPKANIIVDSQYVMG	SEQ ID NO:300
176	NIIVDSQYVMGIITG	SEQ ID NO:301
177	DSQYVMGIITGCPTE	SEQ ID NO:302
178	VMGIITGCPTESESR	SEQ ID NO:303
179	ITGCPTESESRVNO	SEQ ID NO:304
180	PTESESRVNOQIEE	SEQ ID NO:305
181	ESRVNOQIEEEMIKK	SEQ ID NO:306
182	VNOQIEEEMIKKSEIY	SEQ ID NO:307
183	IEEEMIKKSEIYVAWV	SEQ ID NO:308
184	IKKSEIYVAWVPAHK	SEQ ID NO:309
185	EIYVAWVPAHKGIGG	SEQ ID NO:310
186	AWVPAHKGIGGNQEI	SEQ ID NO:311

#	PEPTIDE	SEQUENCE ID
187	AHKGIGGNQEIHLV	SEQ ID NO:312
188	IGGNQEIHLVLSQGI	SEQ ID NO:313
189	QEIHLVLSQGIRQVL	SEQ ID NO:314
190	HLVLSQGIRQVLFLEK	SEQ ID NO:315
191	QGIRQVLFLEKIEPA	SEQ ID NO:316
192	QVLFLEKIEPAQEEH	SEQ ID NO:317
193	LEKIEPAQEEHDKYH	SEQ ID NO:318
194	EPAQEEHDKYHSNVK	SEQ ID NO:319
195	EEHDKYHSNVKELVF	SEQ ID NO:320
196	KYHSNVKELVFKFGL	SEQ ID NO:321
197	NVKELVFKFGLPRIV	SEQ ID NO:322
198	LVFKFGLPRIVARQI	SEQ ID NO:323
199	FGLPRIVARQIVDTC	SEQ ID NO:324
200	RIVARQIVDTC DKCH	SEQ ID NO:325
201	RQIVDTC DKCHQKGE	SEQ ID NO:326
202	DTCDKCHQKGEAIHG	SEQ ID NO:327
203	KCHQKGEAIHGQANS	SEQ ID NO:328
204	KGEAIHGQANS DLGT	SEQ ID NO:329
205	IHGQANS DLGTWQMD	SEQ ID NO:330
206	ANS DLGTWQMDCTHL	SEQ ID NO:331
207	LGTWQMDCTHLEGKI	SEQ ID NO:332
208	QMDCTHLEGKIIIVA	SEQ ID NO:333
209	THLEGKIIIVAVHVA	SEQ ID NO:334
210	GKIIIVAVHVASGFI	SEQ ID NO:335
211	IVAVHVASGFIEAEV	SEQ ID NO:336
212	HVASGFIEAEVIPQE	SEQ ID NO:337
213	GFIEAEVIPQETGRQ	SEQ ID NO:338
214	AEVIPQETGRQTALF	SEQ ID NO:339
215	PQETGRQTALFLLKL	SEQ ID NO:340
216	GRQTALFLLKLGRW	SEQ ID NO:341
217	ALFLLKLGRWPITH	SEQ ID NO:342
218	LKLGRWPITHLHTD	SEQ ID NO:343
219	GRWPITHLHTDNGAN	SEQ ID NO:344
220	ITHLHTDNGANFASQ	SEQ ID NO:345
221	HTDNGANFASQEVKM	SEQ ID NO:346
222	GANFASQEVKMVAWW	SEQ ID NO:347
223	ASQEVKMVAWWAGIE	SEQ ID NO:348
224	VKMVAWWAGIEHTFG	SEQ ID NO:349
225	AWWAGIEHTFGVPYN	SEQ ID NO:350
226	GIEHTFGVPYNPQSQ	SEQ ID NO:351

NO	PEPTIDE	SEQUENCE ID
227	TFGVPYNPQSQGVVE	SEQ ID NO:352
228	PYNPQSQGVVEAMNH	SEQ ID NO:353
229	QSQGVVEAMNHHLKN	SEQ ID NO:354
230	VVEAMNHHLKNQIDR	SEQ ID NO:355
231	MNHHLKNQIDRIREQ	SEQ ID NO:356
232	LKNQIDRIREQANSV	SEQ ID NO:357
233	IDRIREQANSVETIV	SEQ ID NO:358
234	REQANSVETIVLMAV	SEQ ID NO:359
235	NSVETIVLMAVHCMN	SEQ ID NO:360
236	TIVLMAVHCMNFKRR	SEQ ID NO:361
237	MAVHCMNFKRRGGIG	SEQ ID NO:362
238	CMNFKRRGGIGDMTP	SEQ ID NO:363
239	KRRGGIGDMTPAERL	SEQ ID NO:364
240	GIGDMTPAERLINMI	SEQ ID NO:365
241	MTPAERLINMITTEQ	SEQ ID NO:366
242	ERLINMITTEQEIQF	SEQ ID NO:367
243	NMITTEQEIQFQQSK	SEQ ID NO:368
244	TEQEIQFQQSKNSKF	SEQ ID NO:369
245	IQFQQSKNSKFKNFR	SEQ ID NO:370
246	QSKNSKFKNFRVYYR	SEQ ID NO:371
247	SKFKNFRVYYREGRD	SEQ ID NO:372
248	NFRVYYREGRDQLWK	SEQ ID NO:373
249	YYREGRDQLWKGPGE	SEQ ID NO:374
250	GRDQLWKGPGEELLWK	SEQ ID NO:375
251	LWKGPGEELLWKGEA	SEQ ID NO:376
252	PGELLWKGEAVILK	SEQ ID NO:377
253	LWKGEAVILKVGTD	SEQ ID NO:378
254	EGAVILKVGTDIKVV	SEQ ID NO:379
255	ILKVGTDIKVVPRRK	SEQ ID NO:380
256	GTDIKVVPRRKAKII	SEQ ID NO:381
257	KVVPRRKAKIIKDYG	SEQ ID NO:382
258	RRKAKIIKDYGKGKE	SEQ ID NO:383
259	KIIKDYGKGKEVDSS	SEQ ID NO:384
260	DYGGKGKEVDSSSHME	SEQ ID NO:385
261	GKEVDSSSHMEDTGE	SEQ ID NO:386
262	DSSSHMEDTGEAREV	SEQ ID NO:387
263	HMEDTGEAREVA	SEQ ID NO:388

**TABLE 3**

One embodiment of an SIV<sub>mac236</sub> nef peptide pool sequence. Each peptide is 15 amino acids in length and overlaps the preceding peptide by 11 amino acids. The full-length nef sequence [SEQ ID NO:2186] is modified from the HIV sequence database <http://hiv-web.lanl.gov>.

#	PEPTIDE	SEQUENCE ID
1	MGGAISMRRSRPSGD	SEQ ID NO:389
2	ISMRRSRPSGDLRQR	SEQ ID NO:390
3	RSRPSGDLRQRLIRA	SEQ ID NO:391
4	SGDLRQRLLRARGET	SEQ ID NO:392
5	RQRLLRARGETYGR	SEQ ID NO:393
6	LRARGETYGRLLGEV	SEQ ID NO:394
7	GETYGRLLGEVEDGY	SEQ ID NO:395
8	GRLLGEVEDGYSQSP	SEQ ID NO:396
9	GEVEDGYSQSPGGLD	SEQ ID NO:397
10	DGYSQSPGGLDKGLS	SEQ ID NO:398
11	QSPGGLDKGLSSLSC	SEQ ID NO:399
12	GLDKGLSSLSCGQK	SEQ ID NO:400
13	GLSSLSCGQKYNQG	SEQ ID NO:401
14	LSCEGQKYNQGQYMN	SEQ ID NO:402
15	GQKYNQGQYMNTPWR	SEQ ID NO:403
16	NQGQYMNTPWRNP	SEQ ID NO:404
17	YMNTPWRNP	SEQ ID NO:405
18	PWRNP	SEQ ID NO:406
19	PAEEREKLAYRKQNM	SEQ ID NO:407
20	REKLAYRKQNMDDID	SEQ ID NO:408
21	AYRKQNMDDIDE	SEQ ID NO:409



TABLE 4

One embodiment of an SHIV<sub>SF162P3</sub> env peptide pool sequence. Each peptide is 15 amino acids in length and overlaps the preceding peptide by 11 amino acids. Peptide 211 is 14 amino acids in length.

\*Peptide overlaps preceding peptide by 10 amino acids to eliminate a forbidden Q n-terminal peptide.

The full-length env sequence [SEQ ID NO:2187] is modified from the HIV sequence database <http://hiv-web.lanl.gov>.

#	PEPTIDE	SEQUENCE ID	#	PEPTIDE	SEQUENCE ID
1	MRVKGIRKNYQHLWR	SEQ ID NO:410	32	LCVTLHCTNLENATN	SEQ ID NO:441
2	GIRKNYQHLWRGGTL	SEQ ID NO:411	33	LHCTNLENATNTTSS	SEQ ID NO:442
3	NYQHLWRGGTLLLGM	SEQ ID NO:412	34	NLENATNTTSSNWKE	SEQ ID NO:443
4	LWRGGTLLLGMLMIC	SEQ ID NO:413	35	ATNTTSSNWKEMNRG	SEQ ID NO:444
5	GTL LLGMLMICS AVE	SEQ ID NO:414	36	TSSNWKEMNRGEIKN	SEQ ID NO:445
6	LGMLMICS AVEKLWV	SEQ ID NO:415	37	WKEMNRGEIKNCSFN	SEQ ID NO:446
7	MICS AVEKLWVTVYY	SEQ ID NO:416	38	NRGEIKNCSFNVTTS	SEQ ID NO:447
8	AVEKLWVTVYYGVPA	SEQ ID NO:417	39	IKNCSFNVTTSIGNK	SEQ ID NO:448
9	LWVTVYYGVPAWKEA	SEQ ID NO:418	40	SFNVTTSIGNKMQKE	SEQ ID NO:449
10	VYYGVPAWKEATTTL	SEQ ID NO:419	41	TTSIGNKMQKEYALF	SEQ ID NO:450
11	VPAWKEATTTLFCAS	SEQ ID NO:420	42	GNKMQKEYALFYRLD	SEQ ID NO:451
12	KEATTTLFCASDAKA	SEQ ID NO:421	43	MQKEYALFYRLDVVP*	SEQ ID NO:452
13	TTLFCASDAKAYDTE	SEQ ID NO:422	44	YALFYRLDVVPIDND	SEQ ID NO:453
14	CASDAKAYDTEVHNV	SEQ ID NO:423	45	YRLDVVPIDNDNTSY	SEQ ID NO:454
15	AKAYDTEVHNVWATH	SEQ ID NO:424	46	VVPIDNDNTSYNLIN	SEQ ID NO:455
16	DTEVHNVWATHACVP	SEQ ID NO:425	47	DNDNTSYNLINCNTS	SEQ ID NO:456
17	HNWATHACVPTDPN	SEQ ID NO:426	48	TSYNLINCNTSVITQ	SEQ ID NO:457
18	ATHACVPTDPNPQEI	SEQ ID NO:427	49	LINCNTSVITQACPK	SEQ ID NO:458
19	CVPTDPNPQEIVLEN	SEQ ID NO:428	50	NTSVITQACPKVSFE	SEQ ID NO:459
20	DPNPQEIVLENTEN	SEQ ID NO:429	51	ITQACPKVSFEPIPI	SEQ ID NO:460
21	PQEIVLENTENFNM*	SEQ ID NO:430	52	CPKVSFEPIPIHYCA	SEQ ID NO:461
22	VLENTENFNMWKNN	SEQ ID NO:431	53	SFEPIPIHYCAPAGF	SEQ ID NO:462
23	VTENFNMWKNNMVEQ	SEQ ID NO:432	54	IPIHYCAPAGFAILK	SEQ ID NO:463
24	FNMWKNNMVEQMHED	SEQ ID NO:433	55	YCAPAGFAILKCNDK	SEQ ID NO:464
25	KNNMVEQMHEDIISL	SEQ ID NO:434	56	AGFAILKCNDKKFNG	SEQ ID NO:465
26	VEQMHEDIISLWDQS	SEQ ID NO:435	57	ILKCNDKKFNGSGPC	SEQ ID NO:466
27	HEDIISLWDQSLEPC	SEQ ID NO:436	58	NDKKFNGSGPCINVS	SEQ ID NO:467
28	ISLWDQSLEPCVKLT	SEQ ID NO:437	59	FNGSGPCINVSTVQC	SEQ ID NO:468
29	DQSLEPCVKLTPLCV	SEQ ID NO:438	60	GPCINVSTVQCTHGI	SEQ ID NO:469
30	EPCVKLTPLCVTLHC	SEQ ID NO:439	61	NVSTVQCTHGIRPVV	SEQ ID NO:470
31	KLTPLCVTLHCTNLE	SEQ ID NO:440	62	VQCTHGIRPVVSTQL	SEQ ID NO:471

#	PEPTIDE	SEQUENCE ID
63	HGIRPVVSTQLLLNG	SEQ ID NO:472
64	PVVSTQLLLNGSLAE	SEQ ID NO:473
65	TQLLLNGSLAEEGVV	SEQ ID NO:474
66	LNGSLAEEGVVIRSE	SEQ ID NO:475
67	LAEEGVVIRSENFTD	SEQ ID NO:476
68	GVVIRSENFTDNVKT	SEQ ID NO:477
69	RSENFTDNVKTIIIVQ	SEQ ID NO:478
70	FTDNVKTIIIVQLKES	SEQ ID NO:479
71	VKTIIIVQLKESVEIN	SEQ ID NO:480
72	IVQLKESVEINCTRP	SEQ ID NO:481
73	KESVEINCTRPNNT	SEQ ID NO:482
74	EINCTRPNNTNRKSI	SEQ ID NO:483
75	TRPNNTNRKSIPIGP	SEQ ID NO:484
76	NNTRKSIPIGPGKAF	SEQ ID NO:485
77	KSIPIGPGKAFYATG	SEQ ID NO:486
78	IGPGKAFYATGDIIG	SEQ ID NO:487
79	KAFYATGDIIGDIRQ	SEQ ID NO:488
80	ATGDIIGDIRQAHCN	SEQ ID NO:489
81	IIGDIRQAHCNISGE	SEQ ID NO:490
82	IRQAHCNISGEKWN	SEQ ID NO:491
83	HCNISGEKWNNTLKQ	SEQ ID NO:492
84	SGEKWNNTLKQIVTK	SEQ ID NO:493
85	WNNTLKQIVTKLQAO	SEQ ID NO:494
86	LKQIVTKLQAOFENK	SEQ ID NO:495
87	VTKLQAOFENKTIVF	SEQ ID NO:496
88	LQAOFENKTIVFKQS*	SEQ ID NO:497
89	FENKTIVFKQSSGGD	SEQ ID NO:498
90	TIVFKQSSGGDPEIV	SEQ ID NO:499
91	KQSSGGDPEIVMHSF	SEQ ID NO:500
92	GGDPEIVMHSFNCGG	SEQ ID NO:501
93	EIVMHSFNCGGEFFY	SEQ ID NO:502
94	HSFNCGGEFFYCNST	SEQ ID NO:503
95	CGGEFFYCNSTQLFN	SEQ ID NO:504
96	FFYCNSTQLFNSTWN	SEQ ID NO:505
97	NSTQLFNSTWNNTIG	SEQ ID NO:506
98	LFNSTWNNTIGPNNT	SEQ ID NO:507
99	TWNNTIGPNNTNGTI	SEQ ID NO:508
100	TIGPNNTNGTITLPC	SEQ ID NO:509
101	NNTNGTITLPCRIKQ	SEQ ID NO:510
102	GTITLPCRIKQIINR	SEQ ID NO:511

#	PEPTIDE	SEQUENCE ID
103	LPCRIKQIINRWQEV	SEQ ID NO:512
104	IKQIINRWQEVGKAM	SEQ ID NO:513
105	INRWQEVGKAMYAPP	SEQ ID NO:514
106	WQEVGKAMYAPPIRG*	SEQ ID NO:515
107	GKAMYAPPIRGQIRC	SEQ ID NO:516
108	YAPPIRGQIRCSSNI	SEQ ID NO:517
109	IRGQIRCSSNITGLL	SEQ ID NO:518
110	IRCSSNITGLLLTRD	SEQ ID NO:519
111	SNITGLLLTRDGGRE	SEQ ID NO:520
112	GLLLTRDGGREVGNT	SEQ ID NO:521
113	TRDGGREVGNTTEIF	SEQ ID NO:522
114	GREVGNTTEIFRPGG	SEQ ID NO:523
115	GNTTEIFRPGGGDMR	SEQ ID NO:524
116	EIFRPGGGDMRDNWR	SEQ ID NO:525
117	PGGGDMRDNWRSELY	SEQ ID NO:526
118	DMRDNWRSELYKYKV	SEQ ID NO:527
119	NWRSELYKYKVVKIE	SEQ ID NO:528
120	ELYKYKVVKIEPLGV	SEQ ID NO:529
121	YKVVKIEPLGVAPTK	SEQ ID NO:530
122	KIEPLGVAPTKAKRR	SEQ ID NO:531
123	LGVAPTKAKRRVVQR	SEQ ID NO:532
124	PTKAKRRVVQREKRA	SEQ ID NO:533
125	KRRVVQREKRAVTLG	SEQ ID NO:534
126	VQREKRAVTLGAVFL	SEQ ID NO:535
127	KRAVTLGAVFLGFLG	SEQ ID NO:536
128	TLGAVFLGFLGAAGS	SEQ ID NO:537
129	VFLGFLGAAGSTMGA	SEQ ID NO:538
130	FLGAAGSTMGAASLT	SEQ ID NO:539
131	AGSTMGAASLTTLTVQ	SEQ ID NO:540
132	MGAASLTTLTVQARQL	SEQ ID NO:541
133	SLTLTVQARQLLSGI	SEQ ID NO:542
134	TVQARQLLSGIVQQQ	SEQ ID NO:543
135	RQLLSGIVQQQNNLL	SEQ ID NO:544
136	SGIVQQQNNLLRAIE	SEQ ID NO:545
137	VQQQNNLLRAIEAQQ*	SEQ ID NO:546
138	NNLLRAIEAQQRLLQ	SEQ ID NO:547
139	RAIEAQQRLLQLTVW	SEQ ID NO:548
140	AQQRLLQLTVWGIKQ	SEQ ID NO:549
141	LLQLTVWGIKQLQAR	SEQ ID NO:550
142	TVWGIKQLQARVLAV	SEQ ID NO:551

#	PEPTIDE	SEQUENCE ID
143	IKQLQARVLAVERYL	SEQ ID NO:552
144	LQARVLAVERYLKDQ*	SEQ ID NO:553
145	VLAVERYLKDQQLLG	SEQ ID NO:554
146	ERYLKDQQLLGIWGC	SEQ ID NO:555
147	KDQQLLGIWGCSGKL	SEQ ID NO:556
148	LLGIWGCSGKLICTT	SEQ ID NO:557
149	WGCSGKLICTTAVPW	SEQ ID NO:558
150	GKLICTTAVPWNASW	SEQ ID NO:559
151	CTTAVPWNASWSNKS	SEQ ID NO:560
152	VPWNASWSNKSLDQI	SEQ ID NO:561
153	ASWSNKSLDQIWNNM	SEQ ID NO:562
154	NKSLDQIWNNMWME	SEQ ID NO:563
155	DQIWNNMWMEWERE	SEQ ID NO:564
156	NNMTWMEWEREIGNY	SEQ ID NO:565
157	WMEWEREIGNYTNLI	SEQ ID NO:566
158	EREIGNYTNLIYTLI	SEQ ID NO:567
159	GNYTNLIYTLIEESQ	SEQ ID NO:568
160	NLIYTLIEESQNQQE	SEQ ID NO:569
161	TLIEESQNQQEKNEQ	SEQ ID NO:570
162	ESQNQQEKNEQELLE	SEQ ID NO:571
163	NQQEKNEQELLELDK*	SEQ ID NO:572
164	KNEQELLELDKWASL	SEQ ID NO:573
165	ELLELDKWASLWNWL	SEQ ID NO:574
166	LDKWASLWNWLDISK	SEQ ID NO:575
167	ASLWNWLDISKWLWY	SEQ ID NO:576
168	NWLDISKWLWYIKIF	SEQ ID NO:577
169	ISKWLWYIKIFIMIV	SEQ ID NO:578
170	LWYIKIFIMIVGGLV	SEQ ID NO:579
171	KIFIMIVGGLVGLRI	SEQ ID NO:580
172	MIVGGLVGLRIVFTV	SEQ ID NO:581
173	GLVGLRIVFTVLSIV	SEQ ID NO:582
174	LRIVFTVLSIVNRVR	SEQ ID NO:583
175	FTVLSIVNRVRQGYS	SEQ ID NO:584
176	SIVNRVRQGYSPLSF	SEQ ID NO:585
177	RVRQGYSPLSFQTRF	SEQ ID NO:586

#	PEPTIDE	SEQUENCE ID
178	GYSPLSFQTRFPAPR	SEQ ID NO:587
179	LSFQTRFPAPRGLDR	SEQ ID NO:588
180	TRFPAPRGLDRPEGI	SEQ ID NO:589
181	APRGLDRPEGIEEEG	SEQ ID NO:590
182	LDRPEGIEEEEGGERD	SEQ ID NO:591
183	EGIEEEEGGERDRDRS	SEQ ID NO:592
184	EEGGERDRDRSRPLV	SEQ ID NO:593
185	ERDRDRSRPLVHGLL	SEQ ID NO:594
186	DRSRPLVHGLLALIW	SEQ ID NO:595
187	PLVHGLLALIWDDL	SEQ ID NO:596
188	GLLALIWDDLRLSLCL	SEQ ID NO:597
189	LIWDDLRLSLCLFSYH	SEQ ID NO:598
190	DLRLSLCLFSYHRLRD	SEQ ID NO:599
191	LCLFSYHRLRDLILI	SEQ ID NO:600
192	SYHRLRDLILIAARI	SEQ ID NO:601
193	LRDLILIAARIVELL	SEQ ID NO:602
194	ILIAARIVELLGRRG	SEQ ID NO:603
195	ARIVELLGRRGWEAL	SEQ ID NO:604
196	ELLGRRGWEALKYWG	SEQ ID NO:605
197	RRGWEALKYWGNLLQ	SEQ ID NO:606
198	EALKYWGNLLQYWIQ	SEQ ID NO:607
199	YWGNLLQYWIQELKN	SEQ ID NO:608
200	LLQYWIQELKNSAVS	SEQ ID NO:609
201	WIQELKNSAVSLFGA	SEQ ID NO:610
202	LKNSAVSLFGAIAIA	SEQ ID NO:611
203	AVSLFGAIAIAVAEG	SEQ ID NO:612
204	FGAIAIAVAEGTDRI	SEQ ID NO:613
205	AIAVAEGTDRIIEVA	SEQ ID NO:614
206	AEGTDRIIEVAQRIG	SEQ ID NO:615
207	DRIIEVAQRIGRAFL	SEQ ID NO:616
208	EVAQRIGRAFLHIPR	SEQ ID NO:617
209	RIGRAFLHIPRRIRQ	SEQ ID NO:618
210	AFLHIPRRIRQGLER	SEQ ID NO:619
211	IPRRIRQGLERTLL	SEQ ID NO:620



TABLE 5

One embodiment of an HIV-1 consensus B clade Gag peptide pool sequence. Each peptide is 15 amino acids in length and overlaps the preceding peptide by 11 amino acids. Peptide 124 is 12 amino acids in length. The full-length Gag sequence [SEQ ID NO:2188] is modified from the HIV sequence database.

#	PEPTIDE	SEQUENCE ID
1	MGARASVLSGGELDR	SEQ ID NO:621
2	ASVLSGGELDRWEKI	SEQ ID NO:622
3	SGGELDRWEKIRLRP	SEQ ID NO:623
4	LDRWEKIRLRPGGKK	SEQ ID NO:624
5	EKIRLRPGGKKKYKL	SEQ ID NO:625
6	LRPGGKKKYKLKHIV	SEQ ID NO:626
7	GKKKYKLKHIVWASR	SEQ ID NO:627
8	YKLKHIVWASRELER	SEQ ID NO:628
9	HIVWASRELERFAVN	SEQ ID NO:629
10	ASRELERFAVNPGLL	SEQ ID NO:630
11	ELERFAVNPGLLETS	SEQ ID NO:631
12	FAVNPGLLETSEGCR	SEQ ID NO:632
13	PGLLETSEGCRQILG	SEQ ID NO:633
14	ETSEGCRQILGQLQP	SEQ ID NO:634
15	GCRQILGQLQPSLQT	SEQ ID NO:635
16	ILGQLQPSLQTGSEE	SEQ ID NO:636
17	LQPSLQTGSEELRSL	SEQ ID NO:637
18	LQTGSEELRSLYNTV	SEQ ID NO:638
19	SEELRSLYNTVATLY	SEQ ID NO:639
20	RSLYNTVATLYCVHQ	SEQ ID NO:640
21	NTVATLYCVHQRIEV	SEQ ID NO:641
22	TLYCVHQRIEVKDTK	SEQ ID NO:642
23	VHQRIEVKDTKEALE	SEQ ID NO:643
24	IEVKDTKEALEKIEE	SEQ ID NO:644
25	DTKEALEKIEEEQNK	SEQ ID NO:645
26	ALEKIEEEQNKSKKK	SEQ ID NO:646
27	IEEEQNKSKKKAQQA	SEQ ID NO:647
28	QNKSKKKAQQAADT	SEQ ID NO:648
29	KKKAQQAADTGNSS	SEQ ID NO:649
30	QQAADTGNSSQVSQ	SEQ ID NO:650
31	ADTGNSSQVSQNYPI	SEQ ID NO:651
32	NSSQVSQNYPIVQNL	SEQ ID NO:652

#	PEPTIDE	SEQUENCE ID
33	VSQNYPIVQNLQGQM	SEQ ID NO:653
34	YPIVQNLQGQMVHQA	SEQ ID NO:654
35	QNLQGQMVHQAI SPR	SEQ ID NO:655
36	GQMVHQAI SPRTLNA	SEQ ID NO:656
37	HQAI SPRTLNAWVKV	SEQ ID NO:657
38	SPRTLNAWVKVVEEK	SEQ ID NO:658
39	LNAWVKVVEEKAFSP	SEQ ID NO:659
40	VKVVEEKAFSPEVIP	SEQ ID NO:660
41	EKAFSPEVIPMFSA	SEQ ID NO:661
42	FSPEVIPMFSALSEG	SEQ ID NO:662
43	VIPMFSALSEGATPQ	SEQ ID NO:663
44	FSALSEGATPQDLNT	SEQ ID NO:664
45	SEGATPQDLNTMLNT	SEQ ID NO:665
46	TPQDLNTMLNTVGGH	SEQ ID NO:666
47	LNTMLNTVGGHQAAM	SEQ ID NO:667
48	LNTVGGHQAAMQMLK	SEQ ID NO:668
49	GGHQAAMQMLKETIN	SEQ ID NO:669
50	AAMQMLKETINEEAA	SEQ ID NO:670
51	QMLKETINEEAAEWD	SEQ ID NO:671
52	ETINEEAAEWDRLHP	SEQ ID NO:672
53	EEAAEWDRLHPVHAG	SEQ ID NO:673
54	EWDRLHPVHAGPIAP	SEQ ID NO:674
55	LHPVHAGPIAPGQMR	SEQ ID NO:675
56	HAGPIAPGQMRPRG	SEQ ID NO:676
57	IAPGQMRPRGSDIA	SEQ ID NO:677
58	QMRPRGSDIAGTTS	SEQ ID NO:678
59	PRGSDIAGTTSTLQE	SEQ ID NO:679
60	DIAGTTSTLQEQIGW	SEQ ID NO:680
61	TTSTLQEQIGWMTNN	SEQ ID NO:681
62	LQEQIGWMTNNPPIP	SEQ ID NO:682
63	IGWMTNNPPIPVGEI	SEQ ID NO:683
64	TNNPPIPVGEIYKRW	SEQ ID NO:684



#	PEPTIDE	SEQUENCE ID
65	PIPVGEIYKRWIILG	SEQ ID NO:685
66	GEIYKRWIILGLNKI	SEQ ID NO:686
67	KRWIILGLNKIVRMY	SEQ ID NO:687
68	ILGLNKIVRMYSPTS	SEQ ID NO:688
69	NKIVRMYSPTSILDI	SEQ ID NO:689
70	RMYSPTSILDIRQGP	SEQ ID NO:690
71	PTSILDIRQGPKEPF	SEQ ID NO:691
72	LDIRQGPKEPFRDYV	SEQ ID NO:692
73	QGPKEPFRDYVDRFY	SEQ ID NO:693
74	EPFRDYVDRFYKTLR	SEQ ID NO:694
75	DYVDRFYKTLRAEQA	SEQ ID NO:695
76	RFYKTLRAEQASQEV	SEQ ID NO:696
77	TLRAEQASQEVKNWM	SEQ ID NO:697
78	EQASQEVKNWMTETL	SEQ ID NO:698
79	QEVKNWMTETLLVQN	SEQ ID NO:699
80	NWMTETLLVQNaNPD	SEQ ID NO:700
81	ETLLVQNaNPDCKTI	SEQ ID NO:701
82	VQNaNPDCKTILKAL	SEQ ID NO:702
83	NPDCKTILKALGPAA	SEQ ID NO:703
84	KTILKALGPAATLEE	SEQ ID NO:704
85	KALGPAATLEEMMTA	SEQ ID NO:705
86	PAATLEEMMTACQGV	SEQ ID NO:706
87	LEEMMTACQGVGGPG	SEQ ID NO:707
88	MTACQGVGGPGHKAR	SEQ ID NO:708
89	QGVGGPGHKARVLAE	SEQ ID NO:709
90	GPGHKARVLAEAMSQ	SEQ ID NO:710
91	KARVLAEAMSQVTNS	SEQ ID NO:711
92	LAEAMSQVTNSATIM	SEQ ID NO:712
93	MSQVTNSATIMMORG	SEQ ID NO:713
94	TNSATIMMORGNFRN	SEQ ID NO:714
95	TIMMORGNFRNQKRT	SEQ ID NO:715
96	QKGNFRNQKRTVKCF	SEQ ID NO:716
97	FRNQKRTVKCFNCGK	SEQ ID NO:717
98	RKTVKCFNCGKEGHI	SEQ ID NO:718
99	VKCFNCGKEGHIAKN	SEQ ID NO:719
100	NCGKEGHIAKNCRAP	SEQ ID NO:720
101	EGHIAKNCRAPRKKG	SEQ ID NO:721
102	AKNCRAPRKKGCWKC	SEQ ID NO:722
103	RAPRKKGCWKC GKEG	SEQ ID NO:723
104	KKGCWKC GKEGHQMK	SEQ ID NO:724

#	PEPTIDE	SEQUENCE ID
105	WKC GKEGHQMKDCTE	SEQ ID NO:725
106	KEGHQMKDCTERQAN	SEQ ID NO:726
107	QMKDCTERQANFLGK	SEQ ID NO:727
108	CTERQANFLGKIWPS	SEQ ID NO:728
109	QANFLGKIWPSHKGR	SEQ ID NO:729
110	LGKIWPSHKGRPGNF	SEQ ID NO:730
111	WPSHKGRPGNFLQSR	SEQ ID NO:731
112	KGRPGNFLQSRPEPT	SEQ ID NO:732
113	GNFLQSRPEPTAPPE	SEQ ID NO:733
114	QSRPEPTAPPEESFR	SEQ ID NO:734
115	EPTAPPEESFRFGEE	SEQ ID NO:735
116	PPEESFRFGREETTP	SEQ ID NO:736
117	SFRFGREETTPSQKQ	SEQ ID NO:737
118	GEETTPSQKQEPID	SEQ ID NO:738
119	TTTPSQKQEPIDKEL	SEQ ID NO:739
120	SQKQEPIDKELYPLA	SEQ ID NO:740
121	EPIDKELYPLASLRS	SEQ ID NO:741
122	KELYPLASLRS LFGN	SEQ ID NO:742
123	PLASLRS LFGNDPSS	SEQ ID NO:743
124	LRS LFGNDPSSQ	SEQ ID NO:744

TABLE 6

One embodiment of an HIV-1 consensus B clade Nef peptide pool sequence. Each peptide is 15 amino acids in length and overlaps the preceding peptide by 11 amino acids. Peptide 49 is 14 amino acids in length. The full-length Nef sequence [SEQ ID NO:2189] is modified from the HIV sequence database.

#	PEPTIDE	SEQUENCE ID
1	MGGKWSKRSVVGWPT	SEQ ID NO:745
2	WSKRSVVGWPTVRER	SEQ ID NO:746
3	SVVGWPTVRERMRA	SEQ ID NO:747
4	WPTVRERMRAEPAA	SEQ ID NO:748
5	RERMRAEPADGVG	SEQ ID NO:749
6	RRAEPADGVGAVSR	SEQ ID NO:750
7	PAADGVGAVSRDLEK	SEQ ID NO:751
8	GVGAVSRDLEKHGAI	SEQ ID NO:752
9	VSRDLEKHGAITSSN	SEQ ID NO:753
10	LEKHGAITSSNTAAN	SEQ ID NO:754
11	GAITSSNTAANNADC	SEQ ID NO:755
12	SSNTAANNADCAWLE	SEQ ID NO:756
13	AANNADCAWLEAQEE	SEQ ID NO:757
14	ADCAWLEAQEEEEVG	SEQ ID NO:758
15	WLEAQEEEEVGFPVR	SEQ ID NO:759
16	QEEEEVGFPVRPQVP	SEQ ID NO:760
17	EVGFPVRPQVPLRPM	SEQ ID NO:761
18	PVRPQVPLRPMTYKA	SEQ ID NO:762
19	QVPLRPMTYKAAVDL	SEQ ID NO:763
20	RPMTYKAAVDLSHFL	SEQ ID NO:764
21	YKAAVDLSHFLKEKG	SEQ ID NO:765
22	VDLSHFLKEKGGLEG	SEQ ID NO:766
23	HFLKEKGGLEGLIYS	SEQ ID NO:767
24	EKGGGLEGLIYSQKRQ	SEQ ID NO:768
25	LEGLIYSQKRQDILD	SEQ ID NO:769
26	IYSQKRQDILDWVY	SEQ ID NO:770
27	KRQDILDWVYHTQG	SEQ ID NO:771
28	ILDWVYHTQGYFPD	SEQ ID NO:772
29	WVYHTQGYFPDWQNY	SEQ ID NO:773
30	TQGYFPDWQNYTPGP	SEQ ID NO:774
31	FPDWQNYTPGPGIRY	SEQ ID NO:775
32	QNYTPGPGIRYPLTF	SEQ ID NO:776
33	PGPGIRYPLTFGWCF	SEQ ID NO:777

#	PEPTIDE	SEQUENCE ID
34	IRYPLTFGWCFKLVP	SEQ ID NO:778
35	LTFGWCFKLVPVEPE	SEQ ID NO:779
36	WCFKLVPVEPEKVEE	SEQ ID NO:780
37	LVPVEPEKVEEANE	SEQ ID NO:781
38	EPEKVEEANEENNS	SEQ ID NO:782
39	VEEANEENNSLLHP	SEQ ID NO:783
40	NEENNSLLHPMSLH	SEQ ID NO:784
41	NNSLLHPMSLHGMD	SEQ ID NO:785
42	LHPMSLHGMDPERE	SEQ ID NO:786
43	SLHGMDPEREVLVW	SEQ ID NO:787
44	MMDPEREVLVWKFDS	SEQ ID NO:788
45	EREVLVWKFDSRLAF	SEQ ID NO:789
46	LVWKFDSRLAFHHMA	SEQ ID NO:790
47	FDSRLAFHHMARELH	SEQ ID NO:791
48	LAFHHMARELHPEYY	SEQ ID NO:792
49	HMARELHPEYYKDC	SEQ ID NO:793

TABLE 7

One embodiment of an HIV-1 consensus B clade Pol peptide pool sequence. Each peptide is 15 amino acids in length and overlaps the preceding peptide by 11 amino acids. Peptide 248 is 14 amino acids in length. The full-length Pol sequence [SEQ ID NO:2190] is modified from the HIV sequence database HIV-1.

#	PEPTIDE	SEQUENCE ID
1	FFREDLAFPQ GKARE	SEQ ID NO:794
2	DLAFPQ GKAREFSSE	SEQ ID NO:795
3	PQ GKAREFSSEQTRA	SEQ ID NO:796
4	AREFSSEQTRANSPT	SEQ ID NO:797
5	SSEQTRANSPTRREL	SEQ ID NO:798
6	TRANSPTRRELQVWG	SEQ ID NO:799
7	SPTRRELQVWGRDNN	SEQ ID NO:800
8	RELQVWGRDNNLSLSE	SEQ ID NO:801
9	VWGRDNNLSLSEAGAD	SEQ ID NO:802
10	DNNSLSEAGADRQGT	SEQ ID NO:803
11	LSEAGADRQGTVSFS	SEQ ID NO:804
12	GADRQGTVSFSFPQI	SEQ ID NO:805
13	QGTVSFSFPQITLWQ	SEQ ID NO:806
14	SFSFPQITLWQRPLV	SEQ ID NO:807
15	PQITLWQRPLVTIKI	SEQ ID NO:808
16	LWQRPLVTIKIGGQL	SEQ ID NO:809
17	PLVTIKIGGQLKEAL	SEQ ID NO:810
18	IKIGGQLKEALLDTG	SEQ ID NO:811
19	GQLKEALLDTGADDT	SEQ ID NO:812
20	EALLDTGADDTVLEE	SEQ ID NO:813
21	DTGADDTVLEEMNLP	SEQ ID NO:814
22	DDTVLEEMNLPGRWK	SEQ ID NO:815
23	LEEMNLPGRWKPKMI	SEQ ID NO:816
24	NLPGRWKPKMIGGIG	SEQ ID NO:817
25	RWKPKMIGGIGGFIK	SEQ ID NO:818
26	KMIGGIGGFIKVRQY	SEQ ID NO:819
27	GIGGFIKVRQYDQIL	SEQ ID NO:820
28	FIKVRQYDQILIEIC	SEQ ID NO:821
29	RQYDQILIEICGHKA	SEQ ID NO:822
30	QILIEICGHKAIGTV	SEQ ID NO:823
31	EICGHKAIGTVLVGP	SEQ ID NO:824
32	HKAIGTVLVGPTPVN	SEQ ID NO:825

#	PEPTIDE	SEQUENCE ID
33	GTVLVGPTPVNIIGR	SEQ ID NO:826
34	VGPTPVNIIGRNLLT	SEQ ID NO:827
35	PVNIIGRNLLTQIGC	SEQ ID NO:828
36	IGRNLLTQIGCTLNF	SEQ ID NO:829
37	LLTQIGCTLNFPISP	SEQ ID NO:830
38	IGCTLNFPISPIETV	SEQ ID NO:831
39	LNFPISPIETVPVKL	SEQ ID NO:832
40	ISPIETVPVKLKPGM	SEQ ID NO:833
41	ETVPVKLKPGMDGPK	SEQ ID NO:834
42	VKLKPGMDGPKVKQW	SEQ ID NO:835
43	PGMDGPKVKQWPLTE	SEQ ID NO:836
44	GPKVKQWPLTEEEKIK	SEQ ID NO:837
45	KQWPLTEEEKIKALVE	SEQ ID NO:838
46	LTEEEKIKALVEICTE	SEQ ID NO:839
47	KIKALVEICTEMEKE	SEQ ID NO:840
48	LVEICTEMEKEGKIS	SEQ ID NO:841
49	CTEMEKEGKISKIGP	SEQ ID NO:842
50	EKEGKISKIGPENPY	SEQ ID NO:843
51	KISKIGPENPYNTPV	SEQ ID NO:844
52	IGPENPYNTPVFAIK	SEQ ID NO:845
53	NPYNTPVFAIKKKDS	SEQ ID NO:846
54	TPVFAIKKKDKSTKWR	SEQ ID NO:847
55	AIKKKKDKSTKWRKLVD	SEQ ID NO:848
56	KDKSTKWRKLVDREL	SEQ ID NO:849
57	KWRKLVDRELNKRT	SEQ ID NO:850
58	LVDFRELNKRTQDFW	SEQ ID NO:851
59	RELNKRTQDFWEVQL	SEQ ID NO:852
60	KRTQDFWEVQLGIPH	SEQ ID NO:853
61	DFWEVQLGIPHPAGL	SEQ ID NO:854
62	VQLGIPHPAGLKKKK	SEQ ID NO:855
63	IPHPAGLKKKKSVTV	SEQ ID NO:856
64	AGLKKKKSVTVLDVG	SEQ ID NO:857



#	PEPTIDE	SEQUENCE ID
65	KKKSVTVLDVGDAYF	SEQ ID NO:858
66	VTVLDVGDAYFSVPL	SEQ ID NO:859
67	DVGDAYFSVPLDKDF	SEQ ID NO:860
68	AYFSVPLDKDFRKYT	SEQ ID NO:861
69	VPLDKDFRKYTAFTI	SEQ ID NO:862
70	KDFRKYTAFTIPSIN	SEQ ID NO:863
71	KYTAFTIPSINNETP	SEQ ID NO:864
72	FTIPSINNETPGIRY	SEQ ID NO:865
73	SINNETPGIRYQYNV	SEQ ID NO:866
74	ETPGIRYQYNVLPQG	SEQ ID NO:867
75	IRYQYNVLPQGWKGS	SEQ ID NO:868
76	YNVLPQGWKGSPAIF	SEQ ID NO:869
77	PQGWKGSPAIFQSSM	SEQ ID NO:870
78	KGSPAIFQSSMTKIL	SEQ ID NO:871
79	AIFQSSMTKILEPFR	SEQ ID NO:872
80	SSMTKILEPFRKQNP	SEQ ID NO:873
81	KILEPFRKQNPDIVI	SEQ ID NO:874
82	PFRKQNPDIVIYQYM	SEQ ID NO:875
83	QNPDIVIYQYMDDL	SEQ ID NO:876
84	IVIYQYMDDLIVGSD	SEQ ID NO:877
85	QYMDDLIVGSDLEIG	SEQ ID NO:878
86	DLYVGSDLEIGQHRT	SEQ ID NO:879
87	GSDLEIGQHRTKIEE	SEQ ID NO:880
88	EIGQHRTKIEELRQH	SEQ ID NO:881
89	HRTKIEELRQHLLRW	SEQ ID NO:882
90	IEELRQHLLRWGFTT	SEQ ID NO:883
91	RQHLLRWGFTTPDKK	SEQ ID NO:884
92	LRWGFTTPDKKHQKE	SEQ ID NO:885
93	FTTPDKKHQKEPPFL	SEQ ID NO:886
94	DKKHQKEPPFLWMGY	SEQ ID NO:887
95	QKEPPFLWMGYELHP	SEQ ID NO:888
96	PFLWMGYELHPDKWT	SEQ ID NO:889
97	MGYELHPDKWTVQPI	SEQ ID NO:890
98	LHPDKWTVQPIVLPE	SEQ ID NO:891
99	KWTVQPIVLPEKDSW	SEQ ID NO:892
100	QPIVLPEKDSWTVND	SEQ ID NO:893
101	LPEKDSWTVNDIQKL	SEQ ID NO:894
102	DSWTVNDIQKLVGKL	SEQ ID NO:895
103	VNDIQKLVGKLNWAS	SEQ ID NO:896
104	QKLVGKLNWASQIYA	SEQ ID NO:897

#	PEPTIDE	SEQUENCE ID
105	GKLNWASQIYAGIKV	SEQ ID NO:898
106	WASQIYAGIKVKQLC	SEQ ID NO:899
107	IYAGIKVKQLCKLLR	SEQ ID NO:900
108	IKVKQLCKLLRGTKA	SEQ ID NO:901
109	QLCKLLRGTKALTEV	SEQ ID NO:902
110	LLRGTKALTEVIPLT	SEQ ID NO:903
111	TKALTEVIPLTEEA	SEQ ID NO:904
112	TEVIPLTEEALELA	SEQ ID NO:905
113	PLTEEALELAENRE	SEQ ID NO:906
114	EAELELAENREILKE	SEQ ID NO:907
115	ELAENREILKEPVHG	SEQ ID NO:908
116	NREILKEPVHGVYD	SEQ ID NO:909
117	LKEPVHGVYDPSKD	SEQ ID NO:910
118	VHGVYDPSKDLIAE	SEQ ID NO:911
119	YYDPSKDLIAEIQKQ	SEQ ID NO:912
120	SKDLIAEIQKQGQGQ	SEQ ID NO:913
121	IAEIQKQGQGWTYQ	SEQ ID NO:914
122	QKQGQGWTYQIYQE	SEQ ID NO:915
123	QGQWTYQIYQEPFKN	SEQ ID NO:916
124	TYQIYQEPFKNLKTG	SEQ ID NO:917
125	YQEPFKNLKTGKYAR	SEQ ID NO:918
126	FKNLKTGKYARMRGA	SEQ ID NO:919
127	KTGKYARMRGAHTND	SEQ ID NO:920
128	YARMRGAHTNDVKQL	SEQ ID NO:921
129	RGHTNDVKQLTEAV	SEQ ID NO:922
130	TNDVKQLTEAVQKIA	SEQ ID NO:923
131	KQLTEAVQKIATESI	SEQ ID NO:924
132	EAVQKIATESIWIWG	SEQ ID NO:925
133	KIATESIWIWGKTPK	SEQ ID NO:926
134	ESIWIWGKTPKFKLP	SEQ ID NO:927
135	IWGKTPKFKLPIQKE	SEQ ID NO:928
136	TPKFKLPIQKETWEA	SEQ ID NO:929
137	KLPIQKETWEAWWTE	SEQ ID NO:930
138	QKETWEAWWTEYWQA	SEQ ID NO:931
139	WEAWWTEYWQATWIP	SEQ ID NO:932
140	WTEYWQATWIPEWEF	SEQ ID NO:933
141	WQATWIPEWEFVNTP	SEQ ID NO:934
142	WIPEWEFVNTPPLVK	SEQ ID NO:935
143	WEFVNTPPLVKLWYQ	SEQ ID NO:936
144	NTPPLVKLWYQLEKE	SEQ ID NO:937



#	PEPTIDE	SEQUENCE ID
145	LVKLWYQLEKEPIVG	SEQ ID NO:938
146	WYQLEKEPIVGAETF	SEQ ID NO:939
147	EKEPIVGAETFYVDG	SEQ ID NO:940
148	IVGAETFYVDGAANR	SEQ ID NO:941
149	ETFYVDGAANRETKL	SEQ ID NO:942
150	VDGAANRETKLGKAG	SEQ ID NO:943
151	ANRETKLGKAGYVTD	SEQ ID NO:944
152	TKLGKAGYVTDGRGRQ	SEQ ID NO:945
153	KAGYVTDGRGRQKVVS	SEQ ID NO:946
154	VTDGRGRQKVVS LTD T	SEQ ID NO:947
155	GRQKVVS LTD TTNQK	SEQ ID NO:948
156	VVS LTD TTNQKTELQ	SEQ ID NO:949
157	TD TTNQKTELQAIHL	SEQ ID NO:950
158	NQKTELQAIHLALQD	SEQ ID NO:951
159	ELQAIHLALQDSGLE	SEQ ID NO:952
160	IHLALQDSGLEVNIV	SEQ ID NO:953
161	LQDSGLEVNIVTDSQ	SEQ ID NO:954
162	GLEVNIVTDSQYALG	SEQ ID NO:955
163	NIVTDSQYALGIIQA	SEQ ID NO:956
164	DSQYALGIIQAQPK	SEQ ID NO:957
165	ALGIIQAQPKSESE	SEQ ID NO:958
166	IQAQPKSESELVSQ	SEQ ID NO:959
167	PKSESELVSQIIEQ	SEQ ID NO:960
168	ESELVSQIIEQLIKK	SEQ ID NO:961
169	VSQIIEQLIKKEKVY	SEQ ID NO:962
170	IEQLIKKEKVYLAWV	SEQ ID NO:963
171	IKKEKVYLAWVPAHK	SEQ ID NO:964
172	KVYLAWVPAHKGIGG	SEQ ID NO:965
173	AWVPAHKGIGGNEQV	SEQ ID NO:966
174	AHKGIGGNEQVDKLV	SEQ ID NO:967
175	IGGNEQVDKLVSAGI	SEQ ID NO:968
176	EQVDKLVSAGIRKVL	SEQ ID NO:969
177	KLVSAGIRKVLFLDG	SEQ ID NO:970
178	AGIRKVLFLDGIDKA	SEQ ID NO:971
179	KVLFLDGIDKAQEEH	SEQ ID NO:972
180	LDGIDKAQEEHEKYH	SEQ ID NO:973
181	DKAQEEHEKYHSNWR	SEQ ID NO:974
182	EEHEKYHSNWRAMAS	SEQ ID NO:975
183	KYHSNWRAMASDFNL	SEQ ID NO:976
184	NWRAMASDFNLPPVV	SEQ ID NO:977

#	PEPTIDE	SEQUENCE ID
185	MASDFNLPPVVAKEI	SEQ ID NO:978
186	FNLPVVAKEIVASC	SEQ ID NO:979
187	PVVAKEIVASCDKCQ	SEQ ID NO:980
188	KEIVASCDKCQLKGE	SEQ ID NO:981
189	ASCDKCQLKGEAMHG	SEQ ID NO:982
190	KCQLKGEAMHGQVDC	SEQ ID NO:983
191	KGEAMHGQVDCSPGI	SEQ ID NO:984
192	MHGQVDCSPGIWQLD	SEQ ID NO:985
193	VDCSPGIWQLDCTHL	SEQ ID NO:986
194	PGIWQLDCTHLEGKI	SEQ ID NO:987
195	QLDCTHLEGKIILVA	SEQ ID NO:988
196	THLEGKIILVAVHVA	SEQ ID NO:989
197	GKIILVAVHVASGYI	SEQ ID NO:990
198	LVAVHVASGYIEAEV	SEQ ID NO:991
199	HVASGYIEAEVIPAE	SEQ ID NO:992
200	GYIEAEVIPAE TGQE	SEQ ID NO:993
201	AEVIPAE TGQETAYF	SEQ ID NO:994
202	PAETGQETAYFLLKL	SEQ ID NO:995
203	GQETAYFLLKLGRW	SEQ ID NO:996
204	AYFLLKLGRWPVKT	SEQ ID NO:997
205	LKLGRWPVKT IHTD	SEQ ID NO:998
206	GRWPVKT IHTDNGSN	SEQ ID NO:999
207	VKT IHTDNGSNFTST	SEQ ID NO:1000
208	HTDNGSNFTSTTVKA	SEQ ID NO:1001
209	GSNFTSTTVKAACWW	SEQ ID NO:1002
210	TSTTVKAACWWAGIK	SEQ ID NO:1003
211	VKAACWWAGIKQEF	SEQ ID NO:1004
212	CWWAGIKQEF GIPYN	SEQ ID NO:1005
213	GIKQEF GIPYNPQS	SEQ ID NO:1006
214	EF GIPYNPQS QGVVE	SEQ ID NO:1007
215	PYNPQS QGVVES MNK	SEQ ID NO:1008
216	QS QGVVES MNKELKK	SEQ ID NO:1009
217	VVES MNKELKKIIGQ	SEQ ID NO:1010
218	MNKELKKIIGQVRDQ	SEQ ID NO:1011
219	LKKIIGQVRDQAEHL	SEQ ID NO:1012
220	IGQVRDQAEHLKTAV	SEQ ID NO:1013
221	RDQAEHLKTAVQMAV	SEQ ID NO:1014
222	EHLKTAVQMAVFIHN	SEQ ID NO:1015
223	TAVQMAVFIHNFKRK	SEQ ID NO:1016
224	MAVFIHNFKRKGGIG	SEQ ID NO:1017

#	PEPTIDE	SEQUENCE ID
225	IHNFKRKGGIGGYSA	SEQ ID NO:1018
226	KRKGGIGGYSAGERI	SEQ ID NO:1019
227	GIGGYSAGERIVDII	SEQ ID NO:1020
228	YSAGERIVDIIATDI	SEQ ID NO:1021
229	ERIVDIIATDIQTKE	SEQ ID NO:1022
230	DIIATDIQTKELQKQ	SEQ ID NO:1023
231	TDIQTKELOKQITKI	SEQ ID NO:1024
232	TKELQKQITKIQNFR	SEQ ID NO:1025
233	QKQITKIQNFRVYRD	SEQ ID NO:1026
234	TKIQNFRVYRDSRDP	SEQ ID NO:1027
235	NFRVYRDSRDPLWKG	SEQ ID NO:1028
236	YRDSRDPLWKGPAKL	SEQ ID NO:1029
237	RDPLWKGPAKLLWKG	SEQ ID NO:1030
238	WKGPAKLLWKGEAV	SEQ ID NO:1031
239	AKLLWKGEAVVIQD	SEQ ID NO:1032
240	WKGEAVVIQDNSDI	SEQ ID NO:1033
241	GAVVIQDNSDIKVVP	SEQ ID NO:1034
242	IQDNSDIKVVP RRKA	SEQ ID NO:1035
243	SDIKVVP RRKAKIIR	SEQ ID NO:1036
244	VVPRRKAKIIRDY GK	SEQ ID NO:1037
245	RKAKIIRDY GKQ MAG	SEQ ID NO:1038
246	IIRDY GKQ MAGDDCV	SEQ ID NO:1039
247	YGKQ MAGDDCVASRQ	SEQ ID NO:1040
248	MAGDDCVASRQDED	SEQ ID NO:1041

TABLE 8

One embodiment of an HIV-1 consensus B clade Rev peptide pool sequence. Each peptide is 15 amino acids in length and overlaps the preceding peptide by 11 amino acids. Peptide 27 is 13 amino acids in length. The full-length Rev sequence [SEQ ID NO:2191] is modified from the HIV sequence database.

#	PEPTIDE	SEQUENCE ID
1	MAGRSGDSDEELLKTL	SEQ ID NO:1042
2	SGDSDEELLKTVRLIC	SEQ ID NO:1043
3	DEELLKTVRLIKFLYC	SEQ ID NO:1044
4	LKTVRLIKFLYQSNPG	SEQ ID NO:1045
5	RLIKFLYQSNPPSPV	SEQ ID NO:1046
6	FLYQSNPPSPPEGTRQ	SEQ ID NO:1047
7	SNPPSPPEGTRQARRE	SEQ ID NO:1048
8	PSPEGTRQARRNRRR	SEQ ID NO:1049
9	GTRQARRNRRRRWRE	SEQ ID NO:1050
10	ARRNRRRRWRERQRQ	SEQ ID NO:1051
11	RRRRWRERQRQIRSI	SEQ ID NO:1052
12	WRERQRQIRSISEWI	SEQ ID NO:1053
13	QRQIRSISEWILSTY	SEQ ID NO:1054
14	RSISEWILSTYLGRP	SEQ ID NO:1055
15	EWILSTYLGRPAEPV	SEQ ID NO:1056
16	STYLGRPAEPVPLQL	SEQ ID NO:1057
17	GRPAEPVPLQLPPLE	SEQ ID NO:1058
18	EPVPLQLPPLERLTL	SEQ ID NO:1059
19	LQLPPLERLTLDCNE	SEQ ID NO:1060
20	PLERLTLDCNEDCGT	SEQ ID NO:1061
21	TLDCNEDCGTSGTQ	SEQ ID NO:1062
22	NEDCGTSGTQGVGS	SEQ ID NO:1063
23	GTSGTQGVGSPQIL	SEQ ID NO:1064
24	TQGVGSPQILVESP	SEQ ID NO:1065
25	GSPQILVESPVLE	SEQ ID NO:1066
26	ILVESPVLESGTK	SEQ ID NO:1067
27	SPAVLESGTKEE	SEQ ID NO:1068

TABLE 9

One embodiment of an HIV-1 consensus B clade Tat peptide pool sequence. Each peptide is 15 amino acids in length and overlaps the preceding peptide by 11 amino acids. Peptide 24 is 14 amino acids in length. The full-length Tat sequence [SEQ ID NO:2192] is modified from the HIV sequence database.

#	PEPTIDE	SEQUENCE ID
1	MEPVDPRLEPWKHPGP	SEQ ID NO:1069
2	DPRLEPWKHPSQPKP	SEQ ID NO:1070
3	EPWKHPSQPKTACTK	SEQ ID NO:1071
4	HPSQPKTACTNCYCK	SEQ ID NO:1072
5	QPKTACTNCYCKKCC	SEQ ID NO:1073
6	ACTNCYCKKCCFHCQ	SEQ ID NO:1074
7	CYCKKCCFHCQVCFI	SEQ ID NO:1075
8	KCCFHCQVCFITKGL	SEQ ID NO:1076
9	HCQVCFITKGLGISY	SEQ ID NO:1077
10	CFITKGLGISYGRKK	SEQ ID NO:1078
11	KGLGISYGRKKRRQR	SEQ ID NO:1079
12	ISYGRKKRRQRRRAP	SEQ ID NO:1080
13	RKKRRQRRRAPQDSQ	SEQ ID NO:1081
14	RQRRRAPQDSQTHQV	SEQ ID NO:1082
15	RAPQDSQTHQVSLSK	SEQ ID NO:1083
16	DSQTHQVSLSKQPAS	SEQ ID NO:1084
17	HQVSLSKQPASQPRG	SEQ ID NO:1085
18	LSKQPASQPRGDPTG	SEQ ID NO:1086
19	PASQPRGDPTGPKES	SEQ ID NO:1087
20	RGDPTGPKESKKKV	SEQ ID NO:1088
21	TGPKESKKKVERET	SEQ ID NO:1089
22	ESKKKVERETETDP	SEQ ID NO:1090
23	KVERETETDPVDQ	SEQ ID NO:1091



TABLE 10

One embodiment of an HIV-1 consensus B clade Vif peptide pool sequence. Each peptide is 15 amino acids in length and overlaps the preceding peptide by 11 amino acids. Peptide 46 is 12 amino acids in length. The full-length Vif sequence [SEQ ID NO:2193] is modified from the HIV sequence database.

#	PEPTIDE	SEQUENCE ID
1	MENRWQVMIVWQVDR	SEQ ID NO:1092
2	WQVMIVWQVDRMRIR	SEQ ID NO:1093
3	IVWQVDRMRIRTWKS	SEQ ID NO:1094
4	VDRMRIRTWKSLVKH	SEQ ID NO:1095
5	RIRTWKSLVKHHMYI	SEQ ID NO:1096
6	WKSLVKHHMYISRKA	SEQ ID NO:1097
7	VKHHMYISRKAKGWF	SEQ ID NO:1098
8	MYISRKAKGWFYRHH	SEQ ID NO:1099
9	RKAKGWFYRHHYEST	SEQ ID NO:1100
10	GWFYRHHYESTHPRI	SEQ ID NO:1101
11	RHHYESTHPRISSEV	SEQ ID NO:1102
12	ESTHPRISSEVHIPL	SEQ ID NO:1103
13	PRISSEVHIPLGDAR	SEQ ID NO:1104
14	SEVHIPLGDARLVIT	SEQ ID NO:1105
15	IPLGDARLVITTYWG	SEQ ID NO:1106
16	DARLVITTYWGLHTG	SEQ ID NO:1107
17	VITTYWGLHTGERDW	SEQ ID NO:1108
18	YWGLHTGERDWHLGQ	SEQ ID NO:1109
19	HTGERDWHLGQGVSI	SEQ ID NO:1110
20	RDWHLGQGVSIWRK	SEQ ID NO:1111
21	LGQGVSIWRKKRYS	SEQ ID NO:1112
22	VSIEWRKKRYSTQVD	SEQ ID NO:1113
23	WRKKRYSTQVDPDLA	SEQ ID NO:1114
24	RYSTQVDPDLADQLI	SEQ ID NO:1115
25	QVDPDLADQLIHLYY	SEQ ID NO:1116
26	DLADQLIHLYYFDCF	SEQ ID NO:1117
27	QLIHLYYFDCFSESA	SEQ ID NO:1118
28	LYYFDCFSESAIRNA	SEQ ID NO:1119
29	DCFSESAIRNAILGH	SEQ ID NO:1120
30	ESAIRNAILGHIVSP	SEQ ID NO:1121
31	RNAILGHIVSPRCEY	SEQ ID NO:1122
32	LGHIVSPRCEYQAGH	SEQ ID NO:1123
33	VSPRCEYQAGHNKVG	SEQ ID NO:1124

#	PEPTIDE	SEQUENCE ID
34	CEYQAGHNKVGSLQY	SEQ ID NO:1125
35	AGHNKVGSLQYLALA	SEQ ID NO:1126
36	KVGSLQYLALAAALIT	SEQ ID NO:1127
37	LQYLALAAALITPKKI	SEQ ID NO:1128
38	ALAAALITPKKIKPPL	SEQ ID NO:1129
39	LITPKKIKPPLPSVT	SEQ ID NO:1130
40	KKIKPPLPSVTKLTE	SEQ ID NO:1131
41	PPLPSVTKLTEDRWNK	SEQ ID NO:1132
42	PPLPSVTKLTEDRWN	SEQ ID NO:1133
43	SVTKLTEDRWNKPKQK	SEQ ID NO:1134
44	LTEDRWNKPKQTKGH	SEQ ID NO:1135
45	RWNKPKQTKGHRGSH	SEQ ID NO:1136
46	PQKTKGHRGSHTMNG	SEQ ID NO:1137
47	KGHRGSHTMNGH	SEQ ID NO:1138
48	PQKTKGHRGSHTMNGH	SEQ ID NO:1139

TABLE 11

One embodiment of an HIV-1 consensus B clade Vpr peptide pool sequence. Each peptide is 15 amino acids in length and overlaps the preceding peptide by 11 amino acids. Peptide 22 is 12 amino acids in length. The full-length Vpr sequence [SEQ ID NO:2194] is modified from the HIV sequence database.

#	PEPTIDE	SEQUENCE ID
1	MEQAPEDQGPQREPYI	SEQ ID NO:1140
2	PEDQGPQREPYNEWTR	SEQ ID NO:1141
3	GPQREPYNEWLELL	SEQ ID NO:1142
4	EPYNEWLELLEELK	SEQ ID NO:1143
5	EWLELLEELKSEAV	SEQ ID NO:1144
6	ELLEELKSEAVRHFP	SEQ ID NO:1145
7	ELKSEAVRHFPRIWL	SEQ ID NO:1146
8	EAVRHFPRIWLHGLG	SEQ ID NO:1147
9	HFPRIWLHGLGQHIY	SEQ ID NO:1148
10	IWLHGLGQHIYETYG	SEQ ID NO:1149
11	GLGQHIYETYGDTWA	SEQ ID NO:1150
12	HIYETYGDTWAGVEA	SEQ ID NO:1151
13	TYGDTWAGVEAIIRI	SEQ ID NO:1152
14	TWAGVEAIIRILQQL	SEQ ID NO:1153
15	VEAIIRILQQLLFH	SEQ ID NO:1154
16	IRILQQLLFHFRIG	SEQ ID NO:1155
17	QQLLFHFRIGCQHS	SEQ ID NO:1156
18	FIHFRIGCQHSRIGI	SEQ ID NO:1157
19	RIGCQHSRIGITRQR	SEQ ID NO:1158
20	QHSRIGITRQRRARN	SEQ ID NO:1159
21	GITRQRRARNGASR	SEQ ID NO:1160
22	QRRARNGASRS	SEQ ID NO:1161

TABLE 12

One embodiment of an HIV-1 consensus B clade Vpu peptide pool sequence. Each peptide is 15 amino acids in length and overlaps the preceding peptide by 11 amino acids. Peptide 18 is 13 amino acids in length. The full-length Vpu sequence [SEQ ID NO:2195] is modified from the HIV sequence database.

#	PEPTIDE	SEQUENCE ID
1	MQSLQILAIVALVVA	SEQ ID NO:1162
2	QILAIVALVVAIIA	SEQ ID NO:1163
3	IVALVVAIIAIVVW	SEQ ID NO:1164
4	VVAIIAIVVWSIVF	SEQ ID NO:1165
5	IIAIVVWSIVFIEYR	SEQ ID NO:1166
6	VVWSIVFIEYRKILR	SEQ ID NO:1167
7	IVFIEYRKILRQRKI	SEQ ID NO:1168
8	EYRKILRQRKIDRLI	SEQ ID NO:1169
9	ILRQRKIDRLIDRIR	SEQ ID NO:1170
10	RKIDRLIDRIRERAE	SEQ ID NO:1171
11	RLIDRIRERAEDSGN	SEQ ID NO:1172
12	RIRERAEDSGNESEG	SEQ ID NO:1173
13	RAEDSGNESEGDQEE	SEQ ID NO:1174
14	SGNESEGDQEELSAL	SEQ ID NO:1175
15	SEGDQEELSALVEMG	SEQ ID NO:1176
16	QEELSALVEMGHAP	SEQ ID NO:1177
17	SALVEMGHAPWDVD	SEQ ID NO:1178
18	EMGHAPWDVDDL	SEQ ID NO:1179

TABLE 13

One embodiment of a peptide pool sequence of HCV 1a H77. Each peptide is 18 amino acids in length and overlaps the preceding peptide by 11 amino acids. Peptide couples 25 & 26, 153 & 154, 220 & 221, 239 & 240, 242 & 243, 244 & 245, 345 & 346 are divided into 15- and 14-mers due to problematic sequences of the original 18-mer peptide. The full-length HCV 1a H77 sequence [SEQ ID NO:2196] is modified from the HCV sequence database.

#	Peptide	SEQUENCE ID	#	Peptide	SEQUENCE ID
1	MSTNPKPQRKTKRNTNRR	SEQ ID NO:1180	32	SIVYEAADAILHTPGCV	SEQ ID NO:1211
2	QRKTKRNTNRRPQDVKFP	SEQ ID NO:1181	33	DAILHTPGCVPCVREGNA	SEQ ID NO:1212
3	TNRRPQDVKFPGGGQIVG	SEQ ID NO:1182	34	GCVPCVREGNASRCWVAV	SEQ ID NO:1213
4	VKFPGGGQIVGGVYLLPR	SEQ ID NO:1183	35	EGNASRCWVAVTPTVATR	SEQ ID NO:1214
5	QIVGGVYLLPRRGPRLG	SEQ ID NO:1184	36	WVAVTPTVATRDGKLPTT	SEQ ID NO:1215
6	LLPRRGPRLGVRATRKT	SEQ ID NO:1185	37	VATRDGKLPTTQLRRHID	SEQ ID NO:1216
7	RLGVRATRKTSESRQPRG	SEQ ID NO:1186	38	LPTTQLRRHIDLLVGSAT	SEQ ID NO:1217
8	RKTSESRQPRGRROPK	SEQ ID NO:1187	39	RHIDLLVGSATLCSALYV	SEQ ID NO:1218
9	QPRGRROPKARRPEGR	SEQ ID NO:1188	40	GSATLCSALYVGDLCSV	SEQ ID NO:1219
10	PIPKARRPEGRTWAQPGY	SEQ ID NO:1189	41	ALYVGDLCSVFLVGQLF	SEQ ID NO:1220
11	PEGRTWAQPGYPWPLYGN	SEQ ID NO:1190	42	CGSVFLVGQLFTFSPRRH	SEQ ID NO:1221
12	QPGYPWPLYGNEGCGWAG	SEQ ID NO:1191	43	GQLFTFSPRRHWTQDCN	SEQ ID NO:1222
13	LYGNEGCGWAGWLLSPRG	SEQ ID NO:1192	44	PRRHWTQDCNCSIYPGH	SEQ ID NO:1223
14	GWAGWLLSPRGSRPSWGP	SEQ ID NO:1193	45	QDCNCSIYPGHITGHRMA	SEQ ID NO:1224
15	SPRGSRPSWGPTDPRRRS	SEQ ID NO:1194	46	YPGHITGHRMAWDMMNW	SEQ ID NO:1225
16	SWGPTDPRRRSRNLGKVI	SEQ ID NO:1195	47	HRMAWDMMNWSPTAALV	SEQ ID NO:1226
17	RRRSRNLGKVIDTLTCGF	SEQ ID NO:1196	48	MMNWSPTAALVVAQLLRI	SEQ ID NO:1227
18	GKVIDTLTCGFADLMGYI	SEQ ID NO:1197	49	AALVVAQLLRIPQAIMDM	SEQ ID NO:1228
19	TCGFADLMGYIPLVGAPL	SEQ ID NO:1198	50	LLRIPQAIMDMIAGAHWG	SEQ ID NO:1229
20	MGYIPLVGAPLGGAARAL	SEQ ID NO:1199	51	IMDMIAGAHWGVLAGIAY	SEQ ID NO:1230
21	GAPLGGAARALAHGVRVL	SEQ ID NO:1200	52	AHWGVLAGIAYFSMVGNW	SEQ ID NO:1231
22	ARALAHGVRVLEDGVNYA	SEQ ID NO:1201	53	GIAYFSMVGNWAKVLVVL	SEQ ID NO:1232
23	VRVLEDGVNYATGNLPGC	SEQ ID NO:1202	54	VGNWAKVLVVLVLLFAGVD	SEQ ID NO:1233
24	VNYATGNLPGCSFSIFLL	SEQ ID NO:1203	55	LVVLLVLLFAGVDAETHVTG	SEQ ID NO:1234
25	LPGCSFSIFLLALLS	SEQ ID NO:1204	56	AGVDAETHVTGGSAGRTT	SEQ ID NO:1235
26	SFSIFLLALLSCLT	SEQ ID NO:1205	57	HVTGGSAGRTTAGLVGLL	SEQ ID NO:1236
27	IFLLALLSCLTVPASAYQ	SEQ ID NO:1206	58	GRTTAGLVGLLTPGAKQN	SEQ ID NO:1237
28	SCLTVPASAYQVRNSSGL	SEQ ID NO:1207	59	VGLLTPGAKQNIQLINTN	SEQ ID NO:1238
29	SAYQVRNSSGLYHVTNDC	SEQ ID NO:1208	60	AKQNIQLINTNGSWHINS	SEQ ID NO:1239
30	SSGLYHVTNDCPNSSIVY	SEQ ID NO:1209	61	INTNGSWHINSTALNCNE	SEQ ID NO:1240
31	TNDCPNSSIVYEADAIL	SEQ ID NO:1210	62	HINSTALNCNESLNTGWL	SEQ ID NO:1241



#	Peptide	SEQUENCE ID
63	NCNESLNTGWLGLFYQH	SEQ ID NO:1242
64	TGWLGLFYQHKNSSGC	SEQ ID NO:1243
65	FYQHKFNSSGCPERLASC	SEQ ID NO:1244
66	SSGCPERLASCRRLTDF	SEQ ID NO:1245
67	LASCRRLTDFAGWGPI	SEQ ID NO:1246
68	TDFAQGWGPISYANGSGL	SEQ ID NO:1247
69	GPISYANGSGLDERPYCW	SEQ ID NO:1248
70	GSGLDERPYCWHYPPRPC	SEQ ID NO:1249
71	PYCWHYPPRPCGIVPAKS	SEQ ID NO:1250
72	PRPCGIVPAKSVCGPVYC	SEQ ID NO:1251
73	PAKSVCGPVYCFTSPV	SEQ ID NO:1252
74	PVYCFTSPVVGTTDRS	SEQ ID NO:1253
75	SPVVVGTTDRSGAPTYSW	SEQ ID NO:1254
76	TDRSGAPTYSWGANDTDV	SEQ ID NO:1255
77	TYSWGANDTDVFVLNTR	SEQ ID NO:1256
78	DTDVFVLNTRPPLGNWF	SEQ ID NO:1257
79	NNTRPPLGNWFGCTWMNS	SEQ ID NO:1258
80	GNWFGCTWMNSTGFTKVC	SEQ ID NO:1259
81	WMNSTGFTKVCGAPPCVI	SEQ ID NO:1260
82	TKVCGAPPCVIGGVGNNT	SEQ ID NO:1261
83	PCVIGGVGNNTLLCPTDC	SEQ ID NO:1262
84	GNNTLLCPTDCFRKHPEA	SEQ ID NO:1263
85	PTDCFRKHPEATYSRCGS	SEQ ID NO:1264
86	HPEATYSRCGSGPWITPR	SEQ ID NO:1265
87	RCGSGPWITPRCMVDYPY	SEQ ID NO:1266
88	ITPRCMVDYPYRLWHYPC	SEQ ID NO:1267
89	DYPYRLWHYPCNTINYTIF	SEQ ID NO:1268
90	HYPCTINYTIFKVRMYVG	SEQ ID NO:1269
91	YTIFKVRMYVGGVEHRLE	SEQ ID NO:1270
92	MYVGGVEHRLEAACNWTR	SEQ ID NO:1271
93	HRLEAACNWTRGERCDLE	SEQ ID NO:1272
94	NWTRGERCDLEDRDRSEL	SEQ ID NO:1273
95	CDLEDRDRSELSPLLLST	SEQ ID NO:1274
96	RSELSPLLLSTTQWQVLP	SEQ ID NO:1275
97	LLSTTQWQVLPSTFTLP	SEQ ID NO:1276
98	QVLPSTFTLPALSTGLI	SEQ ID NO:1277
99	TTLPALSTGLIHLHQNIV	SEQ ID NO:1278
100	TGLIHLHQNIVDVQYLYG	SEQ ID NO:1279
101	QNIVDVQYLYGVGSSIAS	SEQ ID NO:1280
102	YLYGVGSSIASWAIKWEY	SEQ ID NO:1281

#	Peptide	SEQUENCE ID
103	SIASWAIKWEYVLLFLL	SEQ ID NO:1282
104	KWEYVLLFLLADARVC	SEQ ID NO:1283
105	LFLLLADARVCCLWML	SEQ ID NO:1284
106	ARVCCLWMLLISQAEA	SEQ ID NO:1285
107	WMLLISQEAALLENLVI	SEQ ID NO:1286
108	QEAALLENLVILNAASLA	SEQ ID NO:1287
109	NLVILNAASLAGTHGLVS	SEQ ID NO:1288
110	ASLAGTHGLVSFLVFFCF	SEQ ID NO:1289
111	GLVSFLVFFCFWYLKGR	SEQ ID NO:1290
112	FFCFWYLKGRWVPGAVY	SEQ ID NO:1291
113	LKGRWVPGAVYAFYGMWP	SEQ ID NO:1292
114	GAVYAFYGMWPLLLLLLA	SEQ ID NO:1293
115	GMWPLLLLLLALPQRAYA	SEQ ID NO:1294
116	LLLALPQRAYALDTEVAA	SEQ ID NO:1295
117	RAYALDTEVAASCGGVVL	SEQ ID NO:1296
118	EVAASCGGVVLVGLMALT	SEQ ID NO:1297
119	GVVLVGLMALTLSPYYKR	SEQ ID NO:1298
120	MALTLSPYYKRYISWCMW	SEQ ID NO:1299
121	YKRYISWCMWWLQYFLT	SEQ ID NO:1300
122	WCMWWLQYFLTRVEAQLH	SEQ ID NO:1301
123	YFLTRVEAQLHVWPPLN	SEQ ID NO:1302
124	AQLHVWPPLNVRGGRDA	SEQ ID NO:1303
125	PPLNVRGGRDAVILLMCV	SEQ ID NO:1304
126	GRDAVILLMCVVHPTLVF	SEQ ID NO:1305
127	LMCVVHPTLVFDITKLLL	SEQ ID NO:1306
128	TLVFDITKLLLAIFGPLW	SEQ ID NO:1307
129	KLLLAIFGPLWILQASLL	SEQ ID NO:1308
130	GPLWILQASLLKVPYFVR	SEQ ID NO:1309
131	ASLLKVPYFVRVQGLLRI	SEQ ID NO:1310
132	YFVRVQGLLRICALARKI	SEQ ID NO:1311
133	LLRICALARKIAGGHYVQ	SEQ ID NO:1312
134	ARKIAGGHYVQMAIIKLG	SEQ ID NO:1313
135	HYVQMAIIKLGALTGTIV	SEQ ID NO:1314
136	IKLGALTGTIVYNHLTPL	SEQ ID NO:1315
137	GTIVYNHLTPLRDWAHNG	SEQ ID NO:1316
138	LTPLRDWAHNGLRDLAVA	SEQ ID NO:1317
139	AHNGLRDLAVAVEPVVFS	SEQ ID NO:1318
140	LAVAVEPVVFSRMTKLI	SEQ ID NO:1319
141	VVFSRMTKLITWGADTA	SEQ ID NO:1320
142	TKLITWGADTAACGDIIN	SEQ ID NO:1321

#	Peptide	SEQUENCE ID
143	ADTAACGDIINGLPVSAR	SEQ ID NO:1322
144	DIINGLPVSARRGQEILL	SEQ ID NO:1323
145	VSARRGQEILLGPADGMV	SEQ ID NO:1324
146	EILLGPADGMVSKGWRL	SEQ ID NO:1325
147	DGMVSKGWRLAPITAYA	SEQ ID NO:1326
148	WRLAPITAYAQQTRGLL	SEQ ID NO:1327
149	TAYAQQTRGLLGCIITSL	SEQ ID NO:1328
150	RGLLGCIITSLTGRDKNQ	SEQ ID NO:1329
151	ITSLTGRDKNQVEGEVQI	SEQ ID NO:1330
152	DKNQVEGEVQIVSTATQT	SEQ ID NO:1331
153	EVQIVSTATQTFLAT	SEQ ID NO:1332
154	VSTATQTFLATCIN	SEQ ID NO:1333
155	ATQTFLATCINGVCWTVY	SEQ ID NO:1334
156	TCINGVCWTVYHGAGTRT	SEQ ID NO:1335
157	WTVYHGAGTRTIASPKGP	SEQ ID NO:1336
158	GTRTIASPKGPVIQMYTN	SEQ ID NO:1337
159	PKGPVIQMYTNVDQDLVG	SEQ ID NO:1338
160	MYTNVDQDLVGWPAPQGS	SEQ ID NO:1339
161	DLVGWPAPQGSRLTPCT	SEQ ID NO:1340
162	PQGSRLTPCTCGSSDLY	SEQ ID NO:1341
163	TPCTCGSSDLYLVTRHAD	SEQ ID NO:1342
164	SDLYLVTRHADVIPVRRR	SEQ ID NO:1343
165	RHADVIPVRRRGDSRGS	SEQ ID NO:1344
166	VRRRGDSRGSLLSPRPIS	SEQ ID NO:1345
167	RGSLLSPRPISYLGSSG	SEQ ID NO:1346
168	RPISYLGSSGGPLLCPA	SEQ ID NO:1347
169	GSSGGPLLCPAGHAVGLF	SEQ ID NO:1348
170	LCPAGHAVGLFRAAVCTR	SEQ ID NO:1349
171	VGLFRAAVCTRGVAKAVD	SEQ ID NO:1350
172	VCTRGVAKAVDFIPVENL	SEQ ID NO:1351
173	KAVDFIPVENLETTMRSP	SEQ ID NO:1352
174	VENLETTMRSPVFTDNSS	SEQ ID NO:1353
175	MRSVPFTDNSSPPAVPQS	SEQ ID NO:1354
176	DNSSPPAVPQSFQVAHLH	SEQ ID NO:1355
177	VPQSFQVAHLHAPTGS	SEQ ID NO:1356
178	AHLHAPTGS	SEQ ID NO:1357
179	GSGKSTKVPAAAYAAQGYK	SEQ ID NO:1358
180	VPAAYAAQGYKVLVLNPS	SEQ ID NO:1359
181	QGYKVLVLNPSVAATLGF	SEQ ID NO:1360
182	LNPSVAATLGF	SEQ ID NO:1361

#	Peptide	SEQUENCE ID
183	TLGFGAYMSKAHGVDPN	SEQ ID NO:1362
184	MSKAHGVDPNIRTGVRTI	SEQ ID NO:1363
185	DPNIRTGVRTITTTGSPIT	SEQ ID NO:1364
186	VRTITTTGSPITYSTYK	SEQ ID NO:1365
187	SPITYSTYKFLADGGCS	SEQ ID NO:1366
188	YGKFLADGGCSGGAYDII	SEQ ID NO:1367
189	GGCSGGAYDIIICDECHS	SEQ ID NO:1368
190	YDIIICDECHSTDATSIL	SEQ ID NO:1369
191	ECHSTDATSILGIGTVLD	SEQ ID NO:1370
192	TSILGIGTVLDQAETAG	SEQ ID NO:1371
193	TVLDQAETAGARLVVLAT	SEQ ID NO:1372
194	TAGARLVVLATATPPGSV	SEQ ID NO:1373
195	VLATATPPGSVTVSHPNI	SEQ ID NO:1374
196	PGSVTVSHPNIEEVALST	SEQ ID NO:1375
197	HPNIEEVALSTTGEIPFY	SEQ ID NO:1376
198	ALSTTGEIPFYGKAIPLE	SEQ ID NO:1377
199	IPFYGKAIPLEVIKGRH	SEQ ID NO:1378
200	IPLEVIKGRHLIFCHSK	SEQ ID NO:1379
201	GGRHLIFCHSKKKCDELA	SEQ ID NO:1380
202	CHSKKKCDELA	SEQ ID NO:1381
203	DELA	SEQ ID NO:1382
204	VALGINAVAYYRGLDVS	SEQ ID NO:1383
205	VAYYRGLDVSVIPTSGDV	SEQ ID NO:1384
206	DVSVIPTSGDVVVVSTDA	SEQ ID NO:1385
207	SGDVVVVSTDALMTGFTG	SEQ ID NO:1386
208	STDALMTGFTGDFDSVID	SEQ ID NO:1387
209	GFTGDFDSVIDCNTCVTQ	SEQ ID NO:1388
210	SVIDCNTCVTQTVDFSLD	SEQ ID NO:1389
211	CVTQTVDFSLDPTFTIET	SEQ ID NO:1390
212	FSLDPTFTIETTTLPQDA	SEQ ID NO:1391
213	TIETTTLPQDAVSRTQRR	SEQ ID NO:1392
214	PQDAVSRTQRRGRTGRGK	SEQ ID NO:1393
215	TQRRGRTGRGKPGIYRFV	SEQ ID NO:1394
216	GRGKPGIYRFVAPGERPS	SEQ ID NO:1395
217	YRFVAPGERPSGMFDSSV	SEQ ID NO:1396
218	ERPSGMFDSSVLCECYDA	SEQ ID NO:1397
219	DSSVLCECYDAGCAWYEL	SEQ ID NO:1398
220	CYDAGCAWYELTPAE	SEQ ID NO:1399
221	GCAWYELTPAETTV	SEQ ID NO:1400
222	WYELTPAETTVRLRAYMN	SEQ ID NO:1401

#	Peptide	SEQUENCE ID
223	ETTVRLRAYMNTPLPVC	SEQ ID NO:1402
224	AYMNTPLPVCQDHLEFW	SEQ ID NO:1403
225	LPVCQDHLEFWEGVFTGL	SEQ ID NO:1404
226	LEFWEGVFTGLTHIDAHF	SEQ ID NO:1405
227	FTGLTHIDAHFLSQTQKS	SEQ ID NO:1406
228	DAHFLSQTQKSGENFPYL	SEQ ID NO:1407
229	TKQSGENFPYLVAYQATV	SEQ ID NO:1408
230	FPYLVAYQATVCARAQAP	SEQ ID NO:1409
231	QATVCARAQAPPPSWDQM	SEQ ID NO:1410
232	AQAPPPSWDQMWKCLIRL	SEQ ID NO:1411
233	WDQMWKCLIRLKPTLHGP	SEQ ID NO:1412
234	LIRLKPTLHGPTPLLYRL	SEQ ID NO:1413
235	LHGPTPLLYRLGAVQNEV	SEQ ID NO:1414
236	LYRLGAVQNEVTLTHPIT	SEQ ID NO:1415
237	QNEVTLTHPITKYIMTCM	SEQ ID NO:1416
238	HPITKYIMTCMSADLEV	SEQ ID NO:1417
239	MTCMSADLEVVTST	SEQ ID NO:1418
240	TSTWVLVGGVLAAL	SEQ ID NO:1419
241	WVLVGGVLAALAAAYCLST	SEQ ID NO:1420
242	LAALAAAYCLSTGCVV	SEQ ID NO:1421
243	AAAYCLSTGCVVIVG	SEQ ID NO:1422
244	CLSTGCVVIVGRIVL	SEQ ID NO:1423
245	GCVVIVGRIVLSGK	SEQ ID NO:1424
246	VIVGRIVLSGKPAIIPDR	SEQ ID NO:1425
247	LSGKPAIIPDREVLYQEF	SEQ ID NO:1426
248	IPDREVLYQEFDEMEECS	SEQ ID NO:1427
249	YQEFDEMEECSQHLPYIE	SEQ ID NO:1428
250	EECSQHLPYIEQGMMLAE	SEQ ID NO:1429
251	PYIEQGMMLAEQFKQKAL	SEQ ID NO:1430
252	MLAEQFKQKALGLLQTAS	SEQ ID NO:1431
253	QKALGLLQTASRQAEVIT	SEQ ID NO:1432
254	QTASRQAEVITPAVQTNW	SEQ ID NO:1433
255	EVITPAVQTNWQKLEVFW	SEQ ID NO:1434
256	QTNWQKLEVFWAKHMWNF	SEQ ID NO:1435
257	EVFWAKHMWNFISGIQYL	SEQ ID NO:1436
258	MWNFISGIQYLAGLSTLP	SEQ ID NO:1437
259	IQYLAGLSTLPGNPAIAS	SEQ ID NO:1438
260	STLPGNPAIASLMAFTAA	SEQ ID NO:1439
261	AIASLMAFTAAVTSPLTT	SEQ ID NO:1440
262	FTAAVTSPLTTGQTLLFN	SEQ ID NO:1441

#	Peptide	SEQUENCE ID
263	PLTTGQTLLFNILGGWVA	SEQ ID NO:1442
264	LLFNILGGWVAAQLAAPG	SEQ ID NO:1443
265	GWVAAQLAAPGAATAFVG	SEQ ID NO:1444
266	AAPGAATAFVGAGLAGAA	SEQ ID NO:1445
267	AFVGAGLAGAAIGSVGLG	SEQ ID NO:1446
268	AGAAIGSVGLGKVLVDIL	SEQ ID NO:1447
269	VGLGKVLVDILAGYGAGV	SEQ ID NO:1448
270	VDILAGYGAGVAGALVAF	SEQ ID NO:1449
271	GAGVAGALVAFKIMSGEV	SEQ ID NO:1450
272	LVAFKIMSGEVPSTEDLV	SEQ ID NO:1451
273	SGEVPSTEDLVNLLPAIL	SEQ ID NO:1452
274	EDLVNLLPAILSPGALVV	SEQ ID NO:1453
275	PAILSPGALVVGVCACAI	SEQ ID NO:1454
276	ALVVGVCACAILRRHVGP	SEQ ID NO:1455
277	CAAILRRHVGPGEVAVQW	SEQ ID NO:1456
278	HVGPGEVAVQWMNRLIAF	SEQ ID NO:1457
279	AVQWMNRLIAFASRGNHV	SEQ ID NO:1458
280	LIAFASRGNHVSPTHYVP	SEQ ID NO:1459
281	GNHVSPTHYVPESDAAAR	SEQ ID NO:1460
282	HYVPESDAAARVAILSS	SEQ ID NO:1461
283	AAARVAILSSLTVTQLL	SEQ ID NO:1462
284	ILSSLTVTQLLRRRLHQWI	SEQ ID NO:1463
285	TQLLRRRLHQWISSECTTP	SEQ ID NO:1464
286	HQWISSECTTPCSGSWLR	SEQ ID NO:1465
287	CTTPCSGSWLRDIWDWIC	SEQ ID NO:1466
288	SWLRDIWDWICEVLSDFK	SEQ ID NO:1467
289	DWICEVLSDFKTWLKAKL	SEQ ID NO:1468
290	SDFKTWLKAKLMPQLPGI	SEQ ID NO:1469
291	KAKLMPQLPGIPFVSCQR	SEQ ID NO:1470
292	LPGIPFVSCQRGYRGVWR	SEQ ID NO:1471
293	SCQRGYRGVWRGDGIMHT	SEQ ID NO:1472
294	GVWRGDGIMHTRCHCGAE	SEQ ID NO:1473
295	IMHTRCHCGAEITGHVKN	SEQ ID NO:1474
296	CGAEITGHVKNGTMRIVG	SEQ ID NO:1475
297	HVKNGTMRIVGPRTCRNM	SEQ ID NO:1476
298	RIVGPRTCRNMWSGTFPI	SEQ ID NO:1477
299	CRNMWSGTFPINAYTTGP	SEQ ID NO:1478
300	TFPINAYTTGPCTPLPAP	SEQ ID NO:1479
301	TTGPCTPLPAPNYKFALW	SEQ ID NO:1480
302	LPAPNYKFALWRVSAEEY	SEQ ID NO:1481



#	Peptide	SEQUENCE ID
303	FALWRVSAEEYVEIRRVG	SEQ ID NO:1482
304	AEEYVEIRRVGDFHYVSG	SEQ ID NO:1483
305	RRVGDFHYVSGMTTDLNK	SEQ ID NO:1484
306	YVSGMTTDLNKCPCQIPS	SEQ ID NO:1485
307	DNLKCPCQIPSPEFFTEL	SEQ ID NO:1486
308	QIPSPEFFTELDGVR LHR	SEQ ID NO:1487
309	FTELDGVR LHRFAPPCKP	SEQ ID NO:1488
310	RLHRFAPPCKPLLREEVS	SEQ ID NO:1489
311	PCKPLLREEVSFRVGLHE	SEQ ID NO:1490
312	EEVSFRVGLHEYPVGSQ L	SEQ ID NO:1491
313	GLHEYPVGSQ L PCEPEPD	SEQ ID NO:1492
314	GSQ L PCEPEPDVAVL TSM	SEQ ID NO:1493
315	PEPDVAVL TSM L TDP SHI	SEQ ID NO:1494
316	L TSM L TDP SHI TAE AAGR	SEQ ID NO:1495
317	PSHITAE AAGRRLARGSP	SEQ ID NO:1496
318	AAGRRLARGSPPSMASSS	SEQ ID NO:1497
319	RGSPPSMASSSASQ L SAP	SEQ ID NO:1498
320	ASSSASQ L SAPSLKATCT	SEQ ID NO:1499
321	LSAPSLKATCTANHDSPD	SEQ ID NO:1500
322	ATCTANHDSPDAELIEAN	SEQ ID NO:1501
323	DSPDAELIEANLLWRQEM	SEQ ID NO:1502
324	IEANLLWRQEMGGNITRV	SEQ ID NO:1503
325	RQEMGGNITRVESENKVV	SEQ ID NO:1504
326	ITRVESENKV VILDSFDP	SEQ ID NO:1505
327	NKV VILDSFDP LVAEEDE	SEQ ID NO:1506
328	SFDP LVAEEDE REVS VPA	SEQ ID NO:1507
329	EEDEREVS VPAEILRKSR	SEQ ID NO:1508
330	SVPAEILRKSRRFARALP	SEQ ID NO:1509
331	RKSRRFARALPVWARPDY	SEQ ID NO:1510
332	RALPVWARPDYNPPLVET	SEQ ID NO:1511
333	RPDYNPPLVETWKKPDYE	SEQ ID NO:1512
334	LVETWKKPDYEPPV VHGC	SEQ ID NO:1513
335	PDYEPPV VHGCPLPPPRS	SEQ ID NO:1514
336	VHGCPLPPPRSPPVPPPR	SEQ ID NO:1515
337	PPRSPPVPPPRKKRTVVL	SEQ ID NO:1516
338	PPPRKKRTVVLTESTLST	SEQ ID NO:1517
339	TVVLTESTLSTALAE LAT	SEQ ID NO:1518
340	TLSTALAE LATKSF GSSS	SEQ ID NO:1519
341	ELATKSF GSSSSTSGITGD	SEQ ID NO:1520
342	GSSSSTSGITGDNTTTSSE	SEQ ID NO:1521

#	Peptide	SEQUENCE ID
343	ITGDNTTTSSEPAPSGCP	SEQ ID NO:1522
344	TSSEPAPSGCPPDSDVES	SEQ ID NO:1523
345	SGCPPDSDVESYSSM	SEQ ID NO:1524
346	PDSDVESYSSMPPL	SEQ ID NO:1525
347	DVESYSSMPPLEGEPGDP	SEQ ID NO:1526
348	MPPLEGEPGDPDLSDGSW	SEQ ID NO:1527
349	PGDPDLSDGSWSTVSSGA	SEQ ID NO:1528
350	DGSWSTVSSGADTED	SEQ ID NO:1529
351	TVSSGADTEDVVC	SEQ ID NO:1530
352	SSGADTEDVVCCSMS	SEQ ID NO:1531
353	DTEDVVCCSMSYSW	SEQ ID NO:1532
354	DVVCCSMSYSWTGAL	SEQ ID NO:1533
355	CSMSYSWTGALVTP	SEQ ID NO:1534
356	SYSWTGALVTPCAAEEQK	SEQ ID NO:1535
357	LVTPCAAEEQKL PINALS	SEQ ID NO:1536
358	EEQKL PINALSNSLLRHH	SEQ ID NO:1537
359	NALSNSLLRHHNLVYSTT	SEQ ID NO:1538
360	LRHHNLVYSTT SRSACQR	SEQ ID NO:1539
361	YSTT SRSACQRQKKVTFD	SEQ ID NO:1540
362	ACQRQKKVTFDRLQVLDS	SEQ ID NO:1541
363	VTFDRLQVLDSHYQDVLK	SEQ ID NO:1542
364	VLD SHYQDVLKEVKAAAS	SEQ ID NO:1543
365	DVLKEVKAAASKVKANLL	SEQ ID NO:1544
366	AAASKVKANLLSVEEACS	SEQ ID NO:1545
367	ANLLSVEEACSLTPPHSA	SEQ ID NO:1546
368	EACSLTPPHSAKSKFGYG	SEQ ID NO:1547
369	PHSAKSKFGYGAKDV RCH	SEQ ID NO:1548
370	FGYGAKDV RCHARKAVAH	SEQ ID NO:1549
371	VRCHARKAVAHINSVWKD	SEQ ID NO:1550
372	AVAHINSVWKDLLED SVT	SEQ ID NO:1551
373	VWKDLLED SVTPIDTTIM	SEQ ID NO:1552
374	DSVTPIDTTIMAKNEVFC	SEQ ID NO:1553
375	TTIMAKNEVFCVQPEKGG	SEQ ID NO:1554
376	EVFCVQPEKGGRKPARLI	SEQ ID NO:1555
377	EKGGRKPARLIVFPDLGV	SEQ ID NO:1556
378	ARLIVFPDLGVRVCEKMA	SEQ ID NO:1557
379	DLGVRVCEKMALYDVVSK	SEQ ID NO:1558
380	EKMALYDVVSKLPLAVMG	SEQ ID NO:1559
381	VVSKLPLAVMGSSYGFQY	SEQ ID NO:1560
382	AVMGSSYGFQYSPGQ RVE	SEQ ID NO:1561



#	Peptide	SEQUENCE ID
383	GFQYSPGQORVEFLVQAWK	SEQ ID NO:1562
384	QORVEFLVQAWKSKKTPMG	SEQ ID NO:1563
385	QAWKSKKTPMGFSYDTRC	SEQ ID NO:1564
386	TPMGFSYDTRCFDSTVTE	SEQ ID NO:1565
387	DTRCFDSTVTESDIRTEE	SEQ ID NO:1566
388	TVTESDIRTEEAIIYQCCD	SEQ ID NO:1567
389	RTEEAIIYQCCDLDPQARV	SEQ ID NO:1568
390	QCCDLDPQARVAIKSLTE	SEQ ID NO:1569
391	QARVAIKSLTERLYVGGP	SEQ ID NO:1570
392	SLTERLYVGGPLTNSRGE	SEQ ID NO:1571
393	VGGPLTNSRGENCYRRC	SEQ ID NO:1572
394	SRGENCYRRCRASGVLT	SEQ ID NO:1573
395	YRRCRASGVLTTS CGNTL	SEQ ID NO:1574
396	GVLTTS CGNTLT CYIKAR	SEQ ID NO:1575
397	GNTLT CYIKARAACRAAG	SEQ ID NO:1576
398	IKARAACRAAGLQDCTML	SEQ ID NO:1577
399	RAAGLQDCTMLVCGDDL	SEQ ID NO:1578
400	CTMLVCGDDL VVICESAG	SEQ ID NO:1579
401	DDL VVICESAGVQEDAAS	SEQ ID NO:1580
402	ESAGVQEDAASLRAFTEA	SEQ ID NO:1581
403	DAASLRAFTEAMTRY SAP	SEQ ID NO:1582
404	FTEAMTRY SAPPGDPPQP	SEQ ID NO:1583
405	YSAPPGDPPQP EYDLELI	SEQ ID NO:1584
406	PPQPEYDLELITSCSSNV	SEQ ID NO:1585
407	LELITSCSSNVSV AHDGA	SEQ ID NO:1586
408	SSNVSV AHDGAGKRVYYL	SEQ ID NO:1587
409	HDGAGKRVYYL TRDPTTP	SEQ ID NO:1588
410	VYYL TRDPTTP LARA AWE	SEQ ID NO:1589
411	PTTP LARA AWE TARHTPV	SEQ ID NO:1590
412	AAWE TARHTPV NSWL GNI	SEQ ID NO:1591
413	HTPV NSWL GNI IMFAPTL	SEQ ID NO:1592
414	LGNI IMFAPTL WARMILM	SEQ ID NO:1593
415	APTL WARMILM THFFSVL	SEQ ID NO:1594
416	MILM THFFSVL IARDQLE	SEQ ID NO:1595
417	FSVL IARDQLE QALNCEI	SEQ ID NO:1596
418	DQLE QALNCEI YGACYSI	SEQ ID NO:1597
419	NCEI YGACYSI EPLD	SEQ ID NO:1598
420	YGACYSI EPLD LPP	SEQ ID NO:1599
421	CYSI EPLD LPP I I QRLHG	SEQ ID NO:1600
422	DLPP I I QRLHG LSAFSLH	SEQ ID NO:1601

#	Peptide	SEQUENCE ID
423	RLHGLSAFSLHSYSPGEI	SEQ ID NO:1602
424	FSLHSYSPGEINRVAACL	SEQ ID NO:1603
425	PGEINRVAACLRLKLGVP	SEQ ID NO:1604
426	AACLRLKLGVPPLRAWHR	SEQ ID NO:1605
427	GVPPPLRAWHRARSVRAR	SEQ ID NO:1606
428	WRHRARSVRARLLSRGGR	SEQ ID NO:1607
429	VRARLLSRGGRAAICGKY	SEQ ID NO:1608
430	RGGRAAICGKYLFWAVR	SEQ ID NO:1609
431	CGKYLFWAVR TKLKLTP	SEQ ID NO:1610
432	WAVR TKLKLTP IAAAGRL	SEQ ID NO:1611
433	KLTP IAAAGRL DLSGWFT	SEQ ID NO:1612
434	AGRL DLSGWFT AGYSGGD	SEQ ID NO:1613
435	GWFT AGYSGGDIYHSVSH	SEQ ID NO:1614
436	SGGDIYHSVSHARPRFW	SEQ ID NO:1615
437	SVSHARPRFW FCLLLLA	SEQ ID NO:1616
438	RWFW FCLLLLAAGVG	SEQ ID NO:1617
439	FCLLLLAAGVGIYL	SEQ ID NO:1618
440	LLLAAGVGIYLLPNR	SEQ ID NO:1619

TABLE 14

One embodiment of overlapping 15-mer peptides spanning all proteins of HBV. Genotype A was chosen as the initial HBV strains. Where significant variability in the HBV genome is observed between Genotype A and Genotypes B-D, additional peptides were designed so that the complete set will induce responses to all Genotypes of HBV. Where particular T cell epitopes have been mapped to minimal epitopes, these are also included in the peptide set, to most optimally induce these epitope specific responses. Breakdown of sequences: 1-394 Genotype A sequences – all genes - (Total of 394 peptides); 395-543 Genotypes B/C/D – corresponding to significant variability from Genotype A - (Total of 149 peptides); and 544-564 Known Epitopes (Total of 21 peptides)

#	Peptide	SEQUENCE ID
1	MGGWSSKPRKGMGTN	SEQ ID NO:1620
2	SSKPRKGMGTNLSVP	SEQ ID NO:1621
3	RKGMGTNLSVPNPLG	SEQ ID NO:1622
4	GTNLSVPNPLGFFPD	SEQ ID NO:1623
5	SVPNPLGFFPDHQLD	SEQ ID NO:1624
6	PLGFFPDHQLDPAFG	SEQ ID NO:1625
7	FPDHQLDPAFGANSN	SEQ ID NO:1626
8	QLDPAFGANSNNPDW	SEQ ID NO:1627
9	AFGANSNNPDWDFNP	SEQ ID NO:1628
10	NSNNPDWDFNPIKDH	SEQ ID NO:1629
11	PDWDFNPIKDHWPAA	SEQ ID NO:1630
12	FNPIKDHWPAAQVG	SEQ ID NO:1631
13	KDHWPAAQVGVGAF	SEQ ID NO:1632
14	PAANQVGVGAFGPGL	SEQ ID NO:1633
15	QVGVGAFGPGLTPPH	SEQ ID NO:1634
16	GAFGPGLTPPHGGIL	SEQ ID NO:1635
17	PGLTPPHGGILGWSP	SEQ ID NO:1636
18	PPHGGILGWSPQAQG	SEQ ID NO:1637
19	GILGWSPQAQGILTT	SEQ ID NO:1638
20	WSPQAQGILTTVSTI	SEQ ID NO:1639
21	AQGILTTVSTIPPPA	SEQ ID NO:1640
22	LTTVSTIPPPASTNR	SEQ ID NO:1641
23	STIPPPASTNRQSGR	SEQ ID NO:1642
24	PPASTNRQSGRQPTP	SEQ ID NO:1643
25	TNRQSGRQPTPISPP	SEQ ID NO:1644
26	SGRQPTPISPLRDS	SEQ ID NO:1645
27	PTPISPLRDSHPQA	SEQ ID NO:1646
28	SPPLRDSHPQAMQWN	SEQ ID NO:1647

#	Peptide	SEQUENCE ID
29	RDSHPQAMQWNSTAF	SEQ ID NO:1648
30	PQAMQWNSTAFHQAL	SEQ ID NO:1649
31	QWNSTAFHQALQDPR	SEQ ID NO:1650
32	TAFHQALQDPRVRGL	SEQ ID NO:1651
33	QALQDPRVRGLYLPA	SEQ ID NO:1652
34	DPRVRGLYLPAAGSS	SEQ ID NO:1653
35	RGLYLPAAGSSSGTV	SEQ ID NO:1654
36	LPAGSSSGTVNPAP	SEQ ID NO:1655
37	GSSSGTVNPAPNIAS	SEQ ID NO:1656
38	GTVPNPAPNIASHISS	SEQ ID NO:1657
39	PAPNIASHISSISAR	SEQ ID NO:1658
40	IASHISSISARTGDP	SEQ ID NO:1659
41	ISSISARTGDPVTNM	SEQ ID NO:1660
42	SARTGDPVTNMENIT	SEQ ID NO:1661
43	GDPVTNMENITSGFL	SEQ ID NO:1662
44	TNMENITSGFLGPLL	SEQ ID NO:1663
45	NITSGFLGPLLVLQA	SEQ ID NO:1664
46	GFLGPLLVLQAGFFL	SEQ ID NO:1665
47	PLLVLQAGFFLLTRI	SEQ ID NO:1666
48	LQAGFFLLTRILTIP	SEQ ID NO:1667
49	FFLLTRILTIPQSLD	SEQ ID NO:1668
50	TRILTIPQSLDSWWT	SEQ ID NO:1669
51	TIPQSLDSWWTSLNF	SEQ ID NO:1670
52	SLDSWWTSLNFLGGS	SEQ ID NO:1671
53	WWTSLNFLGGSPVCL	SEQ ID NO:1672
54	LNFLGGSPVCLGQNS	SEQ ID NO:1673
55	GGSPVCLGQNSQSPT	SEQ ID NO:1674
56	VCLGQNSQSPTS NHS	SEQ ID NO:1675

#	Peptide	SEQUENCE ID
57	QNSQSPTSNHSPTSC	SEQ ID NO:1676
58	SPTSNHSPTSCPPIC	SEQ ID NO:1677
59	NHSPTSCPPICPGYR	SEQ ID NO:1678
60	TSCPPICPGYRWMCL	SEQ ID NO:1679
61	PICPGYRWMCLRRFI	SEQ ID NO:1680
62	GYRWMCLRRFIIFLF	SEQ ID NO:1681
63	MCLRRFIIFLLFILL	SEQ ID NO:1682
64	RFIIFLLFILLCLIF	SEQ ID NO:1683
65	FLFILLLLCLIFLLVL	SEQ ID NO:1684
66	LLLCLIFLLVLLDYQ	SEQ ID NO:1685
67	LIFLLVLLDYQGMLP	SEQ ID NO:1686
68	LVLLDYQGMLPVCPL	SEQ ID NO:1687
69	DYQGMLPVCPLIPGS	SEQ ID NO:1688
70	MLPVCPLIPGSTTTS	SEQ ID NO:1689
71	CPLIPGSTTTSTGPC	SEQ ID NO:1690
72	PGSTTTSTGPCKTCT	SEQ ID NO:1691
73	TTSTGPCKTCTTPAQ	SEQ ID NO:1692
74	GPCKTCTTPAQGNSM	SEQ ID NO:1693
75	TCTTPAQGNSMFPS	SEQ ID NO:1694
76	PAQGNSMFPSCCCTK	SEQ ID NO:1695
77	NSMFPSCCCTKPTDG	SEQ ID NO:1696
78	PSCCCTKPTDGNCTC	SEQ ID NO:1697
79	CTKPTDGNCTCIPIP	SEQ ID NO:1698
80	TDGNCTCIPIPSSWA	SEQ ID NO:1699
81	CTCIPIPSSWAFKY	SEQ ID NO:1700
82	PIPSSWAFKYLWEW	SEQ ID NO:1701
83	SWAFKYLWEWASVR	SEQ ID NO:1702
84	AKYLWEWASVRFSWL	SEQ ID NO:1703
85	WEWASVRFSWLSLLV	SEQ ID NO:1704
86	SVRFSWLSLLVPFVQ	SEQ ID NO:1705
87	SWLSLLVPFVQWFVG	SEQ ID NO:1706
88	LLVPFVQWFVGLSPT	SEQ ID NO:1707
89	FVQWFVGLSPTVWLS	SEQ ID NO:1708
90	FVGLSPTVWLSAIWM	SEQ ID NO:1709
91	SPTVWLSAIWMMWYW	SEQ ID NO:1710
92	WLSAIWMMWYWGPSL	SEQ ID NO:1711
93	IWMMWYWGPSLYSIV	SEQ ID NO:1712
94	WYWGPSLYSIVSPFI	SEQ ID NO:1713
95	PSLYSIVSPFIPLLP	SEQ ID NO:1714
96	SIVSPFIPLLPFFC	SEQ ID NO:1715

#	Peptide	SEQUENCE ID
97	PFIFLLPIFFCLWVY	SEQ ID NO:1716
98	FIFLLPIFFCLWVYI	SEQ ID NO:1717
99	MAARLYCQLDPSRDV	SEQ ID NO:1718
100	LYCQLDPSRDVLCRL	SEQ ID NO:1719
101	LDPSRDVLCRLPVGA	SEQ ID NO:1720
102	RDVLCRLPVGAESRG	SEQ ID NO:1721
103	CLRPVGAESRGRPLS	SEQ ID NO:1722
104	VGAESRGRPLSGPLG	SEQ ID NO:1723
105	SRGRPLSGPLGTLSS	SEQ ID NO:1724
106	PLSGPLGTLSSPSPS	SEQ ID NO:1725
107	PLGTLSSPSPSAVPA	SEQ ID NO:1726
108	LSSPSPSAVPADHGA	SEQ ID NO:1727
109	SPSAVPADHGAHLSL	SEQ ID NO:1728
110	VPADHGAHLSLRGLP	SEQ ID NO:1729
111	HGAHLSLRGLPVCAF	SEQ ID NO:1730
112	LSLRGLPVCAFSSAG	SEQ ID NO:1731
113	GLPVCAFSSAGPCAL	SEQ ID NO:1732
114	CAFSSAGPCALRFTS	SEQ ID NO:1733
115	SAGPCALRFTSARCM	SEQ ID NO:1734
116	CALRFTSARCMETTV	SEQ ID NO:1735
117	FTSARCMETTVNAHQ	SEQ ID NO:1736
118	RCMETTVNAHQILPK	SEQ ID NO:1737
119	TTVNAHQILPKVLHK	SEQ ID NO:1738
120	AHQILPKVLHKRTLGL	SEQ ID NO:1739
121	LPKVLHKRTLGLPAM	SEQ ID NO:1740
122	LHKRTLGLPAMSTTD	SEQ ID NO:1741
123	TLGLPAMSTTDLEAY	SEQ ID NO:1742
124	PAMSTTDLEAYFKDC	SEQ ID NO:1743
125	TTDLEAYFKDCVFKD	SEQ ID NO:1744
126	EAYFKDCVFKDWEEL	SEQ ID NO:1745
127	KDCVFKDWEELGEEI	SEQ ID NO:1746
128	FKDWEELGEEIRLMI	SEQ ID NO:1747
129	EELGEEIRLMIFVLG	SEQ ID NO:1748
130	EEIRLMIFVLGGCRH	SEQ ID NO:1749
131	LMIFVLGGCRHKLVC	SEQ ID NO:1750
132	VLGGCRHKLVCAPAP	SEQ ID NO:1751
133	CRHKLVCAPAPCNFF	SEQ ID NO:1752
134	KLVCAPAPCNFF TSA	SEQ ID NO:1753
135	MPLSYQHFRKLLLLLD	SEQ ID NO:1754
136	YQHFRKLLLLLDGTE	SEQ ID NO:1755



#	Peptide	SEQUENCE ID
137	RKLLLLDDGTEAGPL	SEQ ID NO:1756
138	LLDDGTEAGPLEEEL	SEQ ID NO:1757
139	GTEAGPLEEELPRLA	SEQ ID NO:1758
140	GPLEEELPRLADADL	SEQ ID NO:1759
141	EELPRLADADLNRRV	SEQ ID NO:1760
142	RLADADLNRRVAEDL	SEQ ID NO:1761
143	ADLNRRVAEDLNLGN	SEQ ID NO:1762
144	RRVAEDLNLGNLNVS	SEQ ID NO:1763
145	EDLNLGNLNVSIPWT	SEQ ID NO:1764
146	LGNLNVSIPWTHKVG	SEQ ID NO:1765
147	NVSIPWTHKVGNFTG	SEQ ID NO:1766
148	PWTHKVGNFTGLYSS	SEQ ID NO:1767
149	KVGNFTGLYSSTVPI	SEQ ID NO:1768
150	FTGLYSSTVPIFNPE	SEQ ID NO:1769
151	YSSTVPIFNPEWQTP	SEQ ID NO:1770
152	VPIFNPEWQTPSFPK	SEQ ID NO:1771
153	NPEWQTPSFPKIHLO	SEQ ID NO:1772
154	QTPSFPKIHLOEDII	SEQ ID NO:1773
155	FPKIHLOEDIINRCQ	SEQ ID NO:1774
156	HLQEDIINRCQQFVG	SEQ ID NO:1775
157	DIINRCQQFVGPLTV	SEQ ID NO:1776
158	RCQQFVGPLTVNEKR	SEQ ID NO:1777
159	FVGPLTVNEKRRLKL	SEQ ID NO:1778
160	LTVNEKRRLKLIMPA	SEQ ID NO:1779
161	EKRRLKLIMPARFYP	SEQ ID NO:1780
162	LKLIMPARFYPTTKY	SEQ ID NO:1781
163	MPARFYPTTKYLPLD	SEQ ID NO:1782
164	FYPTTKYLPLDKGIK	SEQ ID NO:1783
165	TKYLPLDKGIKPYYP	SEQ ID NO:1784
166	PLDKGIKPYYPDQVV	SEQ ID NO:1785
167	GIKPYYPDQVVNHYF	SEQ ID NO:1786
168	YYPDQVVNHYFQTRH	SEQ ID NO:1787
169	QVVNHYFQTRHYLHT	SEQ ID NO:1788
170	HYFQTRHYLHTLWKA	SEQ ID NO:1789
171	TRHYLHTLWKAGILY	SEQ ID NO:1790
172	LHTLWKAGILYKRET	SEQ ID NO:1791
173	WKAGILYKRETTTSA	SEQ ID NO:1792
174	ILYKRETTTSAFCG	SEQ ID NO:1793
175	RETTTSAFCGSPYS	SEQ ID NO:1794
176	RSASFCGSPYSWEQE	SEQ ID NO:1795

#	Peptide	SEQUENCE ID
177	FCGSPYSWEQELQHG	SEQ ID NO:1796
178	PYSWEQELQHGRLLVI	SEQ ID NO:1797
179	EQELQHGRLLVIKTSQ	SEQ ID NO:1798
180	QHGRLLVIKTSQRHGD	SEQ ID NO:1799
181	LVIKTSQRHGDSEFC	SEQ ID NO:1800
182	TSQRHGDSEFCSQPS	SEQ ID NO:1801
183	HGDSEFCSQPSGILS	SEQ ID NO:1802
184	SFCSQPSGILSRSSV	SEQ ID NO:1803
185	QPSGILSRSSVGPCI	SEQ ID NO:1804
186	ILSRSSVGPCIRSQL	SEQ ID NO:1805
187	SSVGPCIRSQLKQSR	SEQ ID NO:1806
188	PCIRSQLKQSRLLGLQ	SEQ ID NO:1807
189	SQLKQSRLLGLQPHQG	SEQ ID NO:1808
190	QSRLLGLQPHQGPLAS	SEQ ID NO:1809
191	GLQPHQGPLASSQPG	SEQ ID NO:1810
192	HQGPLASSQPGRSGS	SEQ ID NO:1811
193	LASSQPGRSGSIRAR	SEQ ID NO:1812
194	QPGRSGSIRARAHPS	SEQ ID NO:1813
195	SGSIRARAHPSSTRRY	SEQ ID NO:1814
196	RARAHPSSTRRYFGVE	SEQ ID NO:1815
197	HPSTRRYFGVEPSGS	SEQ ID NO:1816
198	RRYFGVEPSGSGHID	SEQ ID NO:1817
199	GVEPSGSGHIDHSVN	SEQ ID NO:1818
200	SGSGHIDHSVNNSSS	SEQ ID NO:1819
201	HIDHSVNNSSSCLHQ	SEQ ID NO:1820
202	SVNNSSSCLHQSAVR	SEQ ID NO:1821
203	SSSCLHQSAVRKAAY	SEQ ID NO:1822
204	LHQSAVRKAAYSHLS	SEQ ID NO:1823
205	AVRKAAYSHLSTSKR	SEQ ID NO:1824
206	AAYSHLSTSKRQSSS	SEQ ID NO:1825
207	HLSTSKRQSSSGHAV	SEQ ID NO:1826
208	SKRQSSSGHAVEFHCL	SEQ ID NO:1827
209	SSSGHAVEFHCLPPS	SEQ ID NO:1828
210	HAVEFHCLPPSSAGS	SEQ ID NO:1829
211	FHCLPPSSAGSQSQG	SEQ ID NO:1830
212	PPSSAGSQSQGSVSS	SEQ ID NO:1831
213	AGSQSQGSVSSCWVL	SEQ ID NO:1832
214	SQGSVSSCWVLQFRN	SEQ ID NO:1833
215	VSSCWVLQFRNSKPC	SEQ ID NO:1834
216	WWLQFRNSKPCSEYC	SEQ ID NO:1835



#	Peptide	SEQUENCE ID
217	FRNSKPCSEYCLSHL	SEQ ID NO:1836
218	KPCSEYCLSHLVNLR	SEQ ID NO:1837
219	EYCLSHLVNLRDWDG	SEQ ID NO:1838
220	SHLVNLRDWDGPCDE	SEQ ID NO:1839
221	NLRDWDGPCDEHGEH	SEQ ID NO:1840
222	DWDGPCDEHGEHHIRI	SEQ ID NO:1841
223	CDEHGEHHIRIPRTP	SEQ ID NO:1842
224	GEHHIRIPRTPARVT	SEQ ID NO:1843
225	IRIPRTPARVTGGVF	SEQ ID NO:1844
226	RTPARVTGGVFLVDK	SEQ ID NO:1845
227	RVTGGVFLVDKNPHN	SEQ ID NO:1846
228	GVFLVDKNPHNTAES	SEQ ID NO:1847
229	VDKNPHNTAESRLVV	SEQ ID NO:1848
230	PHNTAESRLVVDIFSQ	SEQ ID NO:1849
231	AESRLVVDIFSQFSRG	SEQ ID NO:1850
232	LVVDIFSQFSRGITRV	SEQ ID NO:1851
233	FSQFSRGITRVSWPK	SEQ ID NO:1852
234	SRGITRVSWPKFAVP	SEQ ID NO:1853
235	TRVSWPKFAVPNLQS	SEQ ID NO:1854
236	WPKFAVPNLQSLTNL	SEQ ID NO:1855
237	AVPNLQSLTNLLSSN	SEQ ID NO:1856
238	LQSLTNLLSSNLSWL	SEQ ID NO:1857
239	TNLLSSNLSWLSLDV	SEQ ID NO:1858
240	SSNLSWLSLDVSAAF	SEQ ID NO:1859
241	SWLSLDVSAAFYHIP	SEQ ID NO:1860
242	LDVSAAFYHIPLHPA	SEQ ID NO:1861
243	AAFYHIPLHPAAMPH	SEQ ID NO:1862
244	HIPLHPAAMPHLLIG	SEQ ID NO:1863
245	HPAAMPHLLIGSSGL	SEQ ID NO:1864
246	MPHLLIGSSGLSRYV	SEQ ID NO:1865
247	LIGSSGLSRYVARLS	SEQ ID NO:1866
248	SGLSRYVARLSSNSR	SEQ ID NO:1867
249	RYVARLSSNSRINNN	SEQ ID NO:1868
250	RLSSNSRINNNQYGT	SEQ ID NO:1869
251	NSRINNNQYGTMQNL	SEQ ID NO:1870
252	NNNQYGTMQNLHDSC	SEQ ID NO:1871
253	YGTMQNLHDSCSRQL	SEQ ID NO:1872
254	QNLHDSCSRQLYVSL	SEQ ID NO:1873
255	DSCSRQLYVSLMLLY	SEQ ID NO:1874
256	RQLYVSLMLLYKTYG	SEQ ID NO:1875

#	Peptide	SEQUENCE ID
257	VSLMLLYKTYGWKLH	SEQ ID NO:1876
258	LLYKTYGWKLHLYSH	SEQ ID NO:1877
259	TYGWKLHLYSHPIVL	SEQ ID NO:1878
260	KLHLYSHPIVLGFRK	SEQ ID NO:1879
261	YSHPIVLGFRKIPMG	SEQ ID NO:1880
262	IVLGFRKIPMGVGLS	SEQ ID NO:1881
263	FRKIPMGVGLSPFLL	SEQ ID NO:1882
264	PMGVGLSPFLLAQFT	SEQ ID NO:1883
265	GLSPFLLAQFTSAIC	SEQ ID NO:1884
266	FLLAQFTSAICSVVR	SEQ ID NO:1885
267	QFTSAICSVVRRAFP	SEQ ID NO:1886
268	AICSVVRRAFPCLLA	SEQ ID NO:1887
269	VVRRAFPCLLAFSYM	SEQ ID NO:1888
270	AFPCLLAFSYMDVV	SEQ ID NO:1889
271	CLAFSYMDVVLGAK	SEQ ID NO:1890
272	SYMDVVLGAKSVQH	SEQ ID NO:1891
273	DVVLGAKSVQHRESL	SEQ ID NO:1892
274	GAKSVQHRESLYTAV	SEQ ID NO:1893
275	VQHRESLYTAVTNFL	SEQ ID NO:1894
276	ESLYTAVTNFLSLG	SEQ ID NO:1895
277	TAVTNFLSLGLHLN	SEQ ID NO:1896
278	NFLSLGLHLNPNKT	SEQ ID NO:1897
279	SLGLHLNPNKTKRWG	SEQ ID NO:1898
280	HLNPNKTKRWGYSLN	SEQ ID NO:1899
281	NKTKRWGYSLNFMGY	SEQ ID NO:1900
282	RWGYSLNFMGYIIGS	SEQ ID NO:1901
283	SLNFMGYIIGSWGTL	SEQ ID NO:1902
284	MGYIIGSWGTLPODH	SEQ ID NO:1903
285	IGSWGTLPODHIVQK	SEQ ID NO:1904
286	GTLPODHIVQKIKHC	SEQ ID NO:1905
287	QDHIVQKIKHCFRKL	SEQ ID NO:1906
288	VQKIKHCFRKLNVNR	SEQ ID NO:1907
289	KHCFRKLNVNRPIDW	SEQ ID NO:1908
290	RKLPVNRPIDWKVCQ	SEQ ID NO:1909
291	VNRPIDWKVCQRIVG	SEQ ID NO:1910
292	IDWKVCQRIVGLLGF	SEQ ID NO:1911
293	VCQRIVGLLGFAPF	SEQ ID NO:1912
294	IVGLLGFAPFTQCG	SEQ ID NO:1913
295	LGFAAPFTQCGYPAL	SEQ ID NO:1914
296	APFTQCGYPALMPLY	SEQ ID NO:1915

#	Peptide	SEQUENCE ID
297	QCGYPALMPYACIQ	SEQ ID NO:1916
298	PALMPYACIQAKQA	SEQ ID NO:1917
299	PLYACIQAKQAFTFS	SEQ ID NO:1918
300	CIQAKQAFTFSPTYK	SEQ ID NO:1919
301	KQAFTFSPTYKAFLS	SEQ ID NO:1920
302	TFSPTYKAFLSKQYM	SEQ ID NO:1921
303	TYKAFLSKQYMNLYP	SEQ ID NO:1922
304	FLSKQYMNLYPVARQ	SEQ ID NO:1923
305	QYMNLYPVARQRPG	SEQ ID NO:1924
306	LYPVARQRPGLCQVF	SEQ ID NO:1925
307	ARQRPGLCQVFADAT	SEQ ID NO:1926
308	PGLCQVFADATPTGW	SEQ ID NO:1927
309	QVFADATPTGWGLAI	SEQ ID NO:1928
310	DATPTGWGLAIGHQR	SEQ ID NO:1929
311	TGWGLAIGHQMRGT	SEQ ID NO:1930
312	LAIGHQMRGT FVAP	SEQ ID NO:1931
313	HQMRGT FVAPLPIH	SEQ ID NO:1932
314	RGTFVAPLPIHTAEL	SEQ ID NO:1933
315	VAPLPIHTAELLAAC	SEQ ID NO:1934
316	PIHTAELLAACFARS	SEQ ID NO:1935
317	AELLAACFARSRSGA	SEQ ID NO:1936
318	AACFARSRSGAKLIG	SEQ ID NO:1937
319	ARSRSGAKLIGTDNS	SEQ ID NO:1938
320	SGAKLIGTDNSVVL	SEQ ID NO:1939
321	LIGTDNSVVL SRKYT	SEQ ID NO:1940
322	DNSVVL SRKYTSFPW	SEQ ID NO:1941
323	VLSRKYTSFPWLLGC	SEQ ID NO:1942
324	KYTSFPWLLGCTANW	SEQ ID NO:1943
325	FPWLLGCTANWILRG	SEQ ID NO:1944
326	LGCTANWILRGTSFV	SEQ ID NO:1945
327	ANWILRGTSFVYVPS	SEQ ID NO:1946
328	LRGTSFVYVPSALNP	SEQ ID NO:1947
329	SFVYVPSALNPADDP	SEQ ID NO:1948
330	VPSALNPADDP SRGR	SEQ ID NO:1949
331	LNPADDP SRGRLGLS	SEQ ID NO:1950
332	DDPSRGRGLSRPLL	SEQ ID NO:1951
333	RGRGLSRPLLRLPF	SEQ ID NO:1952
334	GLSRPLLRLPFQPTT	SEQ ID NO:1953
335	PLLRLPFQPTTGRTS	SEQ ID NO:1954
336	LPFQPTTGRTSLYAV	SEQ ID NO:1955

#	Peptide	SEQUENCE ID
337	PTTGRTSLYAVSPSV	SEQ ID NO:1956
338	RTSLYAVSPSVPSHL	SEQ ID NO:1957
339	YAVSPSVPSHLPVRV	SEQ ID NO:1958
340	PSVPSHLPVRVHFAS	SEQ ID NO:1959
341	SHLPVRVHFASPLHV	SEQ ID NO:1960
342	VRVHFASPLHVAVWRP	SEQ ID NO:1961
343	RVHFASPLHVAVWRPP	SEQ ID NO:1962
344	MQLFHLCLIISCTCP	SEQ ID NO:1963
345	HLCLIISCTCPTVQA	SEQ ID NO:1964
346	IISCTCPTVQASKLC	SEQ ID NO:1965
347	TCPTVQASKLCLGWL	SEQ ID NO:1966
348	VQASKLCLGWLWGMD	SEQ ID NO:1967
349	KLCLGWLWGMDIDPY	SEQ ID NO:1968
350	GWLWGMDIDPYKEFG	SEQ ID NO:1969
351	GMDIDPYKEFGATVE	SEQ ID NO:1970
352	DPYKEFGATVELLSF	SEQ ID NO:1971
353	EFGATVELLSFLPSD	SEQ ID NO:1972
354	TVELLSFLPSDFFPS	SEQ ID NO:1973
355	LSFLPSDFFPSVRDL	SEQ ID NO:1974
356	PSDFFPSVRDLLDTA	SEQ ID NO:1975
357	FPSVRDLLDTASALY	SEQ ID NO:1976
358	RDLLDTASALYREAL	SEQ ID NO:1977
359	DTASALYREALESPE	SEQ ID NO:1978
360	ALYREALESPEHCSP	SEQ ID NO:1979
361	EALESPEHCSPHHTA	SEQ ID NO:1980
362	SPEHCSPHHTALRQA	SEQ ID NO:1981
363	CSPHHTALRQAILCW	SEQ ID NO:1982
364	HTALRQAILCWGELM	SEQ ID NO:1983
365	RQAILCWGELMTLAT	SEQ ID NO:1984
366	LCWGELMTLATWVGN	SEQ ID NO:1985
367	ELMTLATWVGNNLED	SEQ ID NO:1986
368	LATWVGNNLEDPASR	SEQ ID NO:1987
369	VGNNLEDPASRDLVV	SEQ ID NO:1988
370	LEDPASRDLVVNYVN	SEQ ID NO:1989
371	ASRDLVVNYVNTNMG	SEQ ID NO:1990
372	LVVNYVNTNMG LKIR	SEQ ID NO:1991
373	YVNTNMG LKIRQLLW	SEQ ID NO:1992
374	NMGLKIRQLLWFHIS	SEQ ID NO:1993
375	KIRQLLWFHISCLTF	SEQ ID NO:1994
376	LLWFHISCLTFGRET	SEQ ID NO:1995

#	Peptide	SEQUENCE ID
377	HISCLTFGRETVLEY	SEQ ID NO:1996
378	LTFGRETVLEYLVSF	SEQ ID NO:1997
379	RETVLEYLVSFVWVI	SEQ ID NO:1998
380	LEYLVSFVWVIRTPP	SEQ ID NO:1999
381	VSFGVWIRTPPAYRP	SEQ ID NO:2000
382	VWIRTPPAYRPPNAP	SEQ ID NO:2001
383	TPPAYRPPNAPILST	SEQ ID NO:2002
384	YRPPNAPILSTLPET	SEQ ID NO:2003
385	NAPILSTLPETTVVR	SEQ ID NO:2004
386	LSTLPETTVVRRDR	SEQ ID NO:2005
387	PETTVVRRDRGRSP	SEQ ID NO:2006
388	VVRRDRGRSPRRRT	SEQ ID NO:2007
389	RDRGRSPRRRTSPR	SEQ ID NO:2008
390	RSPRRRTSPRRRRS	SEQ ID NO:2009
391	RRTSPRRRRSQSPR	SEQ ID NO:2010
392	SPRRRRSQSPRRRS	SEQ ID NO:2011
393	RRSQSPRRRRSQSRE	SEQ ID NO:2012
394	QSPRRRRSQSRESQC	SEQ ID NO:2013
395	MGQNLSTSNPLGFFP	SEQ ID NO:2014
396	LDPAFRANTANPDWD	SEQ ID NO:2015
397	NPNKDTWPDANKVGA	SEQ ID NO:2016
398	DTWPDANKVGAGAFG	SEQ ID NO:2017
399	DWDFNPNKDTWPDAN	SEQ ID NO:2018
400	NPNKDHWPANQVGA	SEQ ID NO:2019
401	DHWPEANQVGAGAFG	SEQ ID NO:2020
402	DWDFNPNKDHWPAN	SEQ ID NO:2021
403	NPHKDNWPDANKVGV	SEQ ID NO:2022
404	DNWPDANKVGAGAFG	SEQ ID NO:2023
405	DWDLNPHKDNWPDAN	SEQ ID NO:2024
406	QGILQTLPANPPPAS	SEQ ID NO:2025
407	QTLPANPPPASTNRQ	SEQ ID NO:2026
408	SPQAQGILQTLPANP	SEQ ID NO:2027
409	QGILTTPVPAAPPPAS	SEQ ID NO:2028
410	QPTPLSPPLRDTHPQ	SEQ ID NO:2029
411	LSPPLRNTHPQAMQW	SEQ ID NO:2030
412	NSTTFHQTLQDPRVR	SEQ ID NO:2031
413	GTVPNPVPTTASPISS	SEQ ID NO:2032
414	PVPTTASPISSIFSR	SEQ ID NO:2033
415	TASPISSIFSRIGDP	SEQ ID NO:2034
416	ISSIFSRIGDPALNM	SEQ ID NO:2035

#	Peptide	SEQUENCE ID
417	FSRIGDPALNMENIT	SEQ ID NO:2036
418	GDPALNMENITSGFL	SEQ ID NO:2037
419	GTVSPAQNTVSAISS	SEQ ID NO:2038
420	PAQNTVSAISSILSK	SEQ ID NO:2039
421	TVSAISSILSKTGDP	SEQ ID NO:2040
422	ISSILSKTGDPVPMN	SEQ ID NO:2041
423	LSKTGDPVPMNENIA	SEQ ID NO:2042
424	GDPVPMNENIASGLL	SEQ ID NO:2043
425	NFLGGTTVCLGQNSQ	SEQ ID NO:2044
426	LNFLGGAPTCPGQNS	SEQ ID NO:2045
427	NSQSQISSHSPTCCP	SEQ ID NO:2046
428	QISSHSPTCCPPICP	SEQ ID NO:2047
429	PVCPLLPGTSTTSTG	SEQ ID NO:2048
430	PSSWAFGKFLWEWAS	SEQ ID NO:2049
431	PSSWAFARFLWEWAS	SEQ ID NO:2050
432	WGPSLYSILSPFLPL	SEQ ID NO:2051
433	WGPSLYNILSPFMPL	SEQ ID NO:2052
434	AARVCCQLDPARDVL	SEQ ID NO:2053
435	AARLCCQLDPARDVL	SEQ ID NO:2054
436	RGRPLPGPLGALPPA	SEQ ID NO:2055
437	LPGPLGALPPASPSA	SEQ ID NO:2056
438	LGALPPASPSAVPSD	SEQ ID NO:2057
439	RGRPVSGPFGPLPSP	SEQ ID NO:2058
440	VSGPFGPLPSPSSSA	SEQ ID NO:2059
441	FGPLPSPSSSAVPAD	SEQ ID NO:2060
442	PSPSSSAVPADHGAH	SEQ ID NO:2061
443	SPSAVPTDHGAHLSL	SEQ ID NO:2062
444	TTVNAHRNLPKVLHK	SEQ ID NO:2063
445	AYFKDCVFNEWEELG	SEQ ID NO:2064
446	GEEIRLKVFLGGCR	SEQ ID NO:2065
447	LLLLDDEAGPLEEEL	SEQ ID NO:2066
448	ELPRLADEGLNRRVA	SEQ ID NO:2067
449	VPVFNPWKTPSFNP	SEQ ID NO:2068
450	NIHLHQDIKKCEQF	SEQ ID NO:2069
451	HQDIKKCEQFVGPL	SEQ ID NO:2070
452	IKKCEQFVGPLTVNE	SEQ ID NO:2071
453	NIHLQEDIINRCQQY	SEQ ID NO:2072
454	QEDIINRCQQYVGPL	SEQ ID NO:2073
455	INRCQQYVGPLTVNE	SEQ ID NO:2074
456	QQYVGPLTVNEKRRL	SEQ ID NO:2075



#	Peptide	SEQUENCE ID
457	DIHLQEDIVDRCKQF	SEQ ID NO:2076
458	QEDIVDRCKQFVGPL	SEQ ID NO:2077
459	VDRCKQFVGPLTVNE	SEQ ID NO:2078
460	IKPYYPEHLVNHVFQ	SEQ ID NO:2079
461	WEQELQHGAESFHQQ	SEQ ID NO:2080
462	LQHGAESFHQQSSGI	SEQ ID NO:2081
463	LQHGRLVFQTSTRHG	SEQ ID NO:2082
464	RLVFQTSTRHGDESF	SEQ ID NO:2083
465	QTSTRHGDESFCQS	SEQ ID NO:2084
466	RHGDESFCQSSSGIL	SEQ ID NO:2085
467	SSGILSRPPVGSSLQ	SEQ ID NO:2086
468	LSRPPVGSSLQSKHR	SEQ ID NO:2087
469	PVGSSLQSKHRKSRL	SEQ ID NO:2088
470	SLQSKHRKSRLGLQS	SEQ ID NO:2089
471	KHRKSRLGLQSQQGH	SEQ ID NO:2090
472	SRLGLQSQQGHLARR	SEQ ID NO:2091
473	LQSQQGHLARRQQGR	SEQ ID NO:2092
474	QGHLARRQQGRSWSI	SEQ ID NO:2093
475	ARRQQGRSWSIRAGF	SEQ ID NO:2094
476	QGRSWSIRAGFHPTA	SEQ ID NO:2095
477	WSIRAGFHPTARRPF	SEQ ID NO:2096
478	AGFHPTARRPFGVEP	SEQ ID NO:2097
479	PTARRPFGVEPSGSG	SEQ ID NO:2098
480	RPFGVEPSGSGHTTN	SEQ ID NO:2099
481	VEPSGSGHTTNFASK	SEQ ID NO:2100
482	SGHTTNFASKSASC	SEQ ID NO:2101
483	TTNFASKSASCLYQS	SEQ ID NO:2102
484	ASKSASCLYQSPVRK	SEQ ID NO:2103
485	CIQSQRKSRRLGPQP	SEQ ID NO:2104
486	TQGQLAGRPQGGSGS	SEQ ID NO:2105
487	VEPSGSGHTHNCASS	SEQ ID NO:2106
488	SGHTHNCASSSSSC	SEQ ID NO:2107
489	THNCASSSSSCLHQS	SEQ ID NO:2108
490	LQPQQGSLARGKSGR	SEQ ID NO:2109
491	QGSLARGKSGRSGSI	SEQ ID NO:2110
492	ARGKSGRSGSIRARV	SEQ ID NO:2111
493	SGRSGSIRARVHPTT	SEQ ID NO:2112
494	GSIRARVHPTTRRSF	SEQ ID NO:2113
495	VEPSGSGHIDNSASS	SEQ ID NO:2114
496	SGSGHIDNSASSASSC	SEQ ID NO:2115

#	Peptide	SEQUENCE ID
497	IDNSASSASSCLHQS	SEQ ID NO:2116
498	KAAYPSVSTFEKHSS	SEQ ID NO:2117
499	PSVSTFEKHSSSGHA	SEQ ID NO:2118
500	TFEKHSSSGHVELH	SEQ ID NO:2119
501	KAAYSPISTSKGHSS	SEQ ID NO:2120
502	SPISTSKGHSSSGHA	SEQ ID NO:2121
503	TSKGHSSSGHVELH	SEQ ID NO:2122
504	HVELHNLPPNSARS	SEQ ID NO:2123
505	LHNLPPNSARSQSER	SEQ ID NO:2124
506	PPNSARSQSERPVFP	SEQ ID NO:2125
507	ARSQSERPVFPCWWL	SEQ ID NO:2126
508	SERPVPFPCWWLQFRN	SEQ ID NO:2127
509	VFPCWWLQFRNSKPC	SEQ ID NO:2128
510	HVELHHFPPNSSRS	SEQ ID NO:2129
511	LHHFPPNSSRSQSQG	SEQ ID NO:2130
512	PPNSSRSQSQGSVLS	SEQ ID NO:2131
513	SRSQSQGSVLSCWWL	SEQ ID NO:2132
514	SQGSVLSCWWLQFRN	SEQ ID NO:2133
515	HVELHNIPPSSARS	SEQ ID NO:2134
516	LHNIPPSSARSQSEG	SEQ ID NO:2135
517	PPSSARSQSEGPIFS	SEQ ID NO:2136
518	ARSQSEGPIFSCWWL	SEQ ID NO:2137
519	KPCSDYCLSHIVNLL	SEQ ID NO:2138
520	DYCLSHIVNLLLEDWG	SEQ ID NO:2139
521	SHIVNLLLEDWGPCA	SEQ ID NO:2140
522	SQFSRGNRVSWPKF	SEQ ID NO:2141
523	SQFSRGSTHVSWPKE	SEQ ID NO:2142
524	STSRNINYQHGTMQD	SEQ ID NO:2143
525	NINYQHGTMQDLHDS	SEQ ID NO:2144
526	SNSRIINHQHGTMQN	SEQ ID NO:2145
527	NLYVSLLLLYQTFGR	SEQ ID NO:2146
528	SLLLLYQTFGRKLHL	SEQ ID NO:2147
529	LYQTFGRKLHLYSHP	SEQ ID NO:2148
530	FGRKLHLYSHPIILG	SEQ ID NO:2149
531	SVQHLESLEFTSITNF	SEQ ID NO:2150
532	LESLEFTSITNFLLSL	SEQ ID NO:2151
533	FTSITNFLLSLGIHL	SEQ ID NO:2152
534	YVIGCYGSLPQDHII	SEQ ID NO:2153
535	CYGSLPQDHIIQKIK	SEQ ID NO:2154
536	LPQDHIIQKIKECFR	SEQ ID NO:2155



#	Peptide	SEQUENCE ID
537	QEHIVLKIQCFRKL	SEQ ID NO:2156
538	YKAFLCKQYLNLYPV	SEQ ID NO:2157
539	TPTGWGLVMGHQRM	SEQ ID NO:2158
540	RSRSGANILGTDNSV	SEQ ID NO:2159
541	GRLGLSRPLLRLPFR	SEQ ID NO:2160
542	GRLGLYRPLLHLPFR	SEQ ID NO:2161
543	GRLGLYRPLLRLPYR	SEQ ID NO:2162
544	FLPSDFFPSV	SEQ ID NO:2163
545	VLQAGFFLL	SEQ ID NO:2164
546	FLLTRILTI	SEQ ID NO:2165
547	LLCLIFLLV	SEQ ID NO:2166
548	LLDYQGMLPV	SEQ ID NO:2167
549	WLSLLVPFV	SEQ ID NO:2168
550	LLVPFVQWFV	SEQ ID NO:2169
551	GLSPTVWLSV	SEQ ID NO:2170
552	LLPIFFCLWV	SEQ ID NO:2171
553	YLHTLWKAGI	SEQ ID NO:2172
554	NLSWLSLDV	SEQ ID NO:2173
555	GLSRYVARL	SEQ ID NO:2174
556	KLHLYSHPI	SEQ ID NO:2175
557	LLAQFTSAI	SEQ ID NO:2176
558	YMDDVVLGA	SEQ ID NO:2177
559	YVDDVVLGA	SEQ ID NO:2178
560	YIDDVVLGA	SEQ ID NO:2179
561	FLLSLGIHL	SEQ ID NO:2180
562	ALMPLYACI	SEQ ID NO:2181
563	WILRGTSFV	SEQ ID NO:2182
564	ILRGTSFVYV	SEQ ID NO:2183

## BIBLIOGRAPHY

1. Piot, P., et al., *The global impact of HIV/AIDS*. Nature, 2001. 410(6831): p. 968-73.
2. UNAIDS, *Global estimates of HIV/AIDS epidemic as of end 2002*. 2003, UNAIDS.
3. Borrow, P., et al., *Virus-specific CD8<sup>+</sup> cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection*. J Virol, 1994. 68(9): p. 6103-10.
4. Koup, R.A., et al., *Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome*. J Virol, 1994. 68(7): p. 4650-5.
5. Musey, L., et al., *Cytotoxic-T-cell responses, viral load, and disease progression in early human immunodeficiency virus type 1 infection*. N Engl J Med, 1997. 337(18): p. 1267-74.
6. Ogg, G.S., et al., *Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA*. Science, 1998. 279(5359): p. 2103-6.
7. Carmichael, A., et al., *Quantitative analysis of the human immunodeficiency virus type 1 (HIV-1)-specific cytotoxic T lymphocyte (CTL) response at different stages of HIV-1 infection: differential CTL responses to HIV-1 and Epstein-Barr virus in late disease*. J Exp Med, 1993. 177(2): p. 249-56.
8. Rinaldo, C., et al., *High levels of anti-human immunodeficiency virus type 1 (HIV-1) memory cytotoxic T-lymphocyte activity and low viral load are associated with lack of disease in HIV-1-infected long-term nonprogressors*. J Virol, 1995. 69(9): p. 5838-42.
9. Hay, C.M., et al., *Lack of viral escape and defective in vivo activation of human immunodeficiency virus type 1-specific cytotoxic T lymphocytes in rapidly progressive infection*. J Virol, 1999. 73(7): p. 5509-19.
10. Johnson, P.R., et al., *Inactivated whole SIV vaccine in macaques: evaluation of protective efficacy against challenge with cell-free virus or infected cells*. AIDS Res Hum Retroviruses, 1992. 8(8): p. 1501-5.
11. Cranage, M.P., et al., *Studies on the specificity of the vaccine effect elicited by inactivated simian immunodeficiency virus*. AIDS Res Hum Retroviruses, 1993. 9(1): p. 13-22.
12. Luke, W., et al., *Simian immunodeficiency virus (SIV) gp130 oligomers protect rhesus macaques (*Macaca mulatta*) against the infection with SIVmac32H grown on T-cells or derived ex vivo*. Virology, 1996. 216(2): p. 444-50.

13. Mooij, P., et al., *A clinically relevant HIV-1 subunit vaccine protects rhesus macaques from in vivo passaged simian-human immunodeficiency virus infection*. Aids, 1998. 12(5): p. F15-22.
14. VaxGen, I., *VaxGen Announces Initial Results of its Phase III AIDS Vaccine Trial*. 2003, PR Newswire.
15. Kent, S.J., et al., *Vaccination with attenuated simian immunodeficiency virus by DNA inoculation*. J Virol, 2001. 75(23): p. 11930-4.
16. Kent, S.J., et al., *Enhanced T-cell immunogenicity and protective efficacy of a human immunodeficiency virus type 1 vaccine regimen consisting of consecutive priming with DNA and boosting with recombinant fowlpox virus*. J Virol, 1998. 72(12): p. 10180-8.
17. Santra, S., et al., *Prior vaccination increases the epitopic breadth of the cytotoxic T-lymphocyte response that evolves in rhesus monkeys following a simian-human immunodeficiency virus infection*. J Virol, 2002. 76(12): p. 6376-81.
18. Estcourt, M.J., et al., *Prime-boost immunization generates a high frequency, high-avidity CD8(+) cytotoxic T lymphocyte population*. Int Immunol, 2002. 14(1): p. 31-7.
19. Marzo, A.L., et al., *Tumor-specific CD4+ T cells have a major "post-licensing" role in CTL mediated anti-tumor immunity*. J Immunol, 2000. 165(11): p. 6047-55.
20. Nelson, D.J., et al., *Tumor progression despite efficient tumor antigen cross-presentation and effective "arming" of tumor antigen-specific CTL*. J Immunol, 2001. 166(9): p. 5557-66.
21. Ritchie, D.S., et al., *Dendritic cell elimination as an assay of cytotoxic T lymphocyte activity in vivo*. J Immunol Methods, 2000. 246(1-2): p. 109-17.
22. Carbone, F.R. and M.J. Bevan, *Induction of ovalbumin-specific cytotoxic T cells by in vivo peptide immunization*. J Exp Med, 1989. 169(3): p. 603-12.
23. Carbone, F.R., et al., *Induction of cytotoxic T lymphocytes by primary in vitro stimulation with peptides*. J Exp Med, 1988. 167(6): p. 1767-79.
24. Kast, W.M., et al., *Protection against lethal Sendai virus infection by in vivo priming of virus-specific cytotoxic T lymphocytes with a free synthetic peptide*. Proc Natl Acad Sci U S A, 1991. 88(6): p. 2283-7.
25. Schulz, M., R.M. Zinkernagel, and H. Hengartner, *Peptide-induced antiviral protection by cytotoxic T cells*. Proc Natl Acad Sci U S A, 1991. 88(3): p. 991-3.
26. Feltkamp, M.C., et al., *Vaccination with cytotoxic T lymphocyte epitope-containing peptide protects against a tumor induced by human papillomavirus type 16-transformed cells*. Eur J Immunol, 1993. 23(9): p. 2242-9.

27. Celluzzi, C.M., et al., *Peptide-pulsed dendritic cells induce antigen-specific CTL-mediated protective tumor immunity*. J Exp Med, 1996. 183(1): p. 283-7.
28. Kearney, E.R., et al., *Visualization of peptide-specific T cell immunity and peripheral tolerance induction in vivo*. Immunity, 1994. 1(4): p. 327-39.
29. Aichele, P., et al., *T cell priming versus T cell tolerance induced by synthetic peptides*. J Exp Med, 1995. 182(1): p. 261-6.
30. Toes, R.E., et al., *Enhanced tumor outgrowth after peptide vaccination. Functional deletion of tumor-specific CTL induced by peptide vaccination can lead to the inability to reject tumors*. J Immunol, 1996. 156(10): p. 3911-8.
31. Toes, R.E., et al., *Peptide vaccination can lead to enhanced tumor growth through specific T-cell tolerance induction*. Proc Natl Acad Sci U S A, 1996. 93(15): p. 7855-60.
32. Dale, C.J., et al., *Chimeric human papilloma virus-simian/human immunodeficiency virus virus-like-particle vaccines: immunogenicity and protective efficacy in macaques*. Virology, 2002. 301(1): p. 176-87.
33. Jin, X., et al., *Dramatic rise in plasma viremia after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques*. J Exp Med, 1999. 189(6): p. 991-8.
34. Carbone, F.R. and M.J. Bevan, *Class I-restricted processing and presentation of exogenous cell-associated antigen in vivo*. J Exp Med, 1990. 171(2): p. 377-87.
35. Larsson, M., et al., *Efficiency of cross presentation of vaccinia virus-derived antigens by human dendritic cells*. Eur J Immunol, 2001. 31(12): p. 3432-42.
36. Chen, Q., et al., *A direct comparison of cytolytic T-lymphocyte responses to Melan-A peptides in vitro: differential immunogenicity of Melan-A27-35 and Melan-A26-35*. Melanoma Res, 2000. 10(1): p. 16-25.
37. Lu, W., et al., *Therapeutic dendritic-cell vaccine for simian AIDS*. Nat Med, 2003. 9(1): p. 27-32.
38. Pospisilova, D., et al., *Generation of functional dendritic cells for potential use in the treatment of acute lymphoblastic leukemia*. Cancer Immunol Immunother, 2002. 51(2): p. 72-8.
39. Melief, C.J., et al., *Effective therapeutic anticancer vaccines based on precision guiding of cytolytic T lymphocytes*. Immunol Rev, 2002. 188(1): p. 177-82.
40. Mehlhop, E., et al., *Enhanced in vitro stimulation of rhesus macaque dendritic cells for activation of SIV-specific T cell responses*. J Immunol Methods, 2002. 260(1-2): p. 219-34.



41. Sapp, M., et al., *Dendritic cells generated from blood monocytes of HIV-1 patients are not infected and act as competent antigen presenting cells eliciting potent T-cell responses*. Immunol Lett, 1999. 66(1-3): p. 121-8.
42. Chougnet, C., et al., *Normal immune function of monocyte-derived dendritic cells from HIV-infected individuals: implications for immunotherapy*. J Immunol, 1999. 163(3): p. 1666-73.